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Testing “good genes” hypothesis using experimental and meta-analytic approach

PhD thesis prepared under the supervision

of

Professor Jacek Radwan

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„Należę do tych, którzy myślą, że nauka jest rzeczą wielkiego piękna”

(“I am among those who think that science has great beauty”)

Maria Skłodowska-Curie

Mojej Babci, Urszuli Dąmbskiej-Prokop, i Dziadkowi, Janowi Prokopowi
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Last but not least, I want to thank everyone I should have thanked in this section but forgot – which is due to the fact that I happen to forget my own name what with the stress and hustle of these last days before submitting the thesis. I'm sorry – and thank you!

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Abstract

Female preferences for particular values of male morphological, behavioral or chemical traits have been documented in many animal taxa, but the question about evolutionary mechanisms responsible for their origin and maintenance remains unanswered. The good genes hypothesis proposes that female choice targets those traits that signal genetic quality of males and thus preferences evolve because the progeny of choosy females inherits high fitness-conferring alleles from the fathers. This hypothesis, first formulated over thirty years ago, has stimulated a huge amount of research work in the field of sexual selection, but still remains a matter of controversy, with two crucial issues unresolved so far: whether female choice indeed leads to enhanced offspring fitness and what are the mechanisms maintaining genetic variation in male sexual traits.

In my PhD, I attempted to test several predictions of the good genes hypothesis.

I used a meta-analytic approach to summarize the results of studies measuring associations between male attractiveness-related traits and offspring fitness components, thereby trying to evaluate existing evidence for the good genes effects of female choice (Chapter 3). I found a moderate and highly significant positive correlation ($r = 0.235$, $p < 0.001$), calculated across a range of different sire and offspring traits in 35 animal species, and corrected for publication bias. This suggests that on average, genetic correlation between male attractiveness and fitness is likely to be positive, which is a prerequisite for the good genes selection on female preferences to operate.

In the first two chapters of this thesis, I describe the results of experiments on stalk-eyed flies (*Teleopsis dalmanni*), designed to test the genic capture hypothesis, which offers both a theoretical solution to the puzzle of the maintenance of genetic variation in male sexual traits and a mechanism by which a genetic correlation between these traits and fitness could arise. This hypothesis links the expression of sexual traits in males to the number of deleterious mutations in their genomes. In Chapter 1, I describe an indirect test of genic capture predictions, performed by analyzing the inbreeding depression in male sexual ornament (eyespan), whereas in Chapter 2 I tested the hypothesis directly, by manipulating the level of mutation load with varying levels of ionizing radiation and measuring the response in male eyespan. In both chapters, non-sexual morphological traits were also measured for comparison and in Chapter 2, I additionally measured two life history traits (male fertility and female fecundity). I found no support for genic capture hypothesis in the results of those experiments. The magnitude of inbreeding depression in male eyespan was low, fitting within the range characterizing morphological traits (Chapter 1). The level of mutation load had no influence on male eyespan, in contrast with the effect on life history traits which tended to decline with increasing levels of load, but consistently with the (lack of) response in non-sexual morphological traits (Chapter 2).

In the fourth chapter, I focus on the hypothesis that genetic quality of a male, or more precisely – of his gametes, decreases with age due to the accumulation of germline mutations

and therefore, females should prefer to mate with young males. In bulb mite *Rhizoglyphus robini*, previous research has shown that old males sire worse quality daughters than young males do. In Chapter 4 of this thesis, I found evidence for female discrimination against mating with older partners.

"I remember well the time when the thought of the eye made me cold all over, but I have got over this stage of the complaint (…). The sight of a feather in a peacock's tail, whenever I gaze at it, makes me sick!"

Charles Darwin

"Sex transforms life."

Mary Jane West-Eberhard

General Introduction

Sexual selection is considered to be one of the most pervasive forces directing evolution (Kotiaho and Puurtinen 2007). It occurs when individuals (in most mating systems – males) compete for access to gametes of the opposite sex (Andersson 1994), either directly (intra-sexual selection) or by trying to "excite or charm those of the opposite sex, generally the females, which no longer remain passive, but select the most agreeable partners" (Darwin 1871) (inter-sexual competition). Female preferences for specific male phenotypes have been documented for a wide range of taxa (Andersson 1994). In many species, they have led to the evolution of elaborate morphological, behavioral or chemical secondary sexual traits in males. The mechanisms responsible for the origin and maintenance of female preferences have been debated ever since Darwin (1871) and still remain to be satisfactorily explained (Kokko et al. 2003; Kokko et al. 2006). Indicator models of mate choice evolution propose that traits determining attractiveness of males reflect their quality and the benefits of mating with high quality males create a positive selection on female preferences. Those benefits can be direct (*e.g.* fertility assurance, nutrients, parental care or protection) or/and indirect (alleles increasing offspring fitness). The evolution of female choice for direct benefits is conceptually rather simple and empirically well supported (Jones and Ratterman 2009). However, female preferences have also been recorded in species where males do not appear to provide their mates with anything but sperm (Andersson 1994), where preferred males offer reduced direct benefits (less paternal care) (Moller and Jennions 2001) or inflict increased direct costs (Pitnick and Garcia-Gonzalez 2002). Indirect benefits models have been invoked to explain the origin and maintenance of female choice behavior in such systems. Those models propose that preference alleles can spread and persist in populations because they are in linkage disequilibrium with other alleles that are directly and positively related to fitness (Kirkpatrick and Barton 1997).

In my PhD, I attempted to test several predictions of the good genes hypothesis, the oldest and most widely discussed of the indirect benefits hypotheses. According to good genes models, female preferences evolve because preferred male traits are indicators of genetic

quality (Zahavi 1975) or more precisely, breeding value for fitness (Tomkins et al. 2004, Hunt et al. 2004) and therefore, progeny of choosy females inherits high-fitness alleles from their fathers. Despite *ca.* three decades of research inspired by this hypothesis, two crucial issues remain unresolved: (1) how is genetic variation in male sexual traits maintained? and (2) does female choice indeed increase offspring fitness?

The first issue has been known as the paradox of the lek (e.g. Taylor and Williams 1982): persistent female preferences for particular male trait values create a directional selection that is expected to deplete genetic variance in the affected male traits, thereby removing any benefits mate choice could initially be conferring. However, the preferences persist (Andersson 1994) and apparently, so does the genetic variation in male secondary sexual traits (Pomiankowski and Moller 1995). A variety of mechanisms that could potentially contribute to the maintenance of this variation have been proposed (Radwan 2008) and the problem remains a subject of much debate. The second issue concerns the most basic prediction of the good genes hypothesis and has been a subject of a bulk of research (for references, see Appendix). In the first two chapters of this thesis, I report on the results of experiments on stalk-eyed flies (*Teleopsis dalmanni*) designed to test the genic capture hypothesis (Rowe and Houle 1996, Tomkins et al. 2004), which offers both a theoretical solution for the puzzle of the maintenance of genetic variation in male sexual traits and a mechanism by which a genetic correlation between these traits and fitness could arise. The hypothesis assumes that expression of male sexual traits depends on condition, defined as defined as a pool of resources available for competing life-history traits (Andersson 1986; Rowe and Houle 1996), which in turn will depend on the efficiency of acquiring and processing those resources from the environment. Condition is likely to be highly polygenic, and hence its genetic variance may be maintained under mutation-selection balance. The expression of sexual traits is therefore predicted to reflect mutation load. In Chapter 1, I describe an indirect test of this prediction, based on the fact that deleterious mutations are considered the main cause of inbreeding depression (Charlesworth and Charlesworth 1999). Therefore, if sexual ornaments are indeed condition-dependent as predicted by the genic capture hypothesis, they should be particularly sensitive to inbreeding. In the second chapter, I test for genic capture directly, by manipulating mutation load with varying levels of ionizing radiation and measuring the response in ornamental and non-ornamental traits.

In the third chapter, I use a meta-analytic approach to summarize the results of extant studies measuring the relationships between male attractiveness-related traits and offspring fitness components, thereby trying to assess the average magnitude of the abovementioned correlation across different animal taxa.

Given that variation in genetic quality between males is thought to result largely from differences in their mutation load (Charlesworth and Hughes 1999) it is worth noting that such load can be expected to vary with age (Brooks and Kemp 2001). In the last chapter, I focus on

the hypothesis that genetic quality of a male, or more precisely – of his gametes, decreases with age due to the accumulation of germline mutations (Drost and Lee 1995; Hansen and Price 1999) and therefore, females should prefer to mate with young males. In bulb mite *Rhizoglyphus robini*, previous research has shown that old males sire daughters that are less fecund than those of younger males (Prokop et al. 2007). Therefore, in the fourth chapter of this thesis, I attempt to test for female discrimination against old partners and for the presence of a 'trade up' behavior (re-mating decisions conditional on the quality of both the previous and potential new partner, Jennions and Petrie 2000) with respect to male age.

Chapter 1

Low inbreeding depression in a sexual trait in the stalk-eyed fly *Teleopsis dalmanni*.

Note:

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Introduction

Female preferences for elaborate male sexual traits have been documented for a number of species in which males contribute only gametes to the next generation (Andersson 1994). In such systems, mate choice has been hypothesised to benefit females genetically. Such indirect benefits may arise because sons of choosy females inherit genes for elaborate traits from the attractive fathers (Fisher 1930), and/or because progeny of both sexes inherit "good genes" (Zahavi 1975). This latter benefit can arise if the costs of sexual ornaments ensure that only males possessing genes for effective resource acquisition can afford their elaboration (Zahavi 1975; Getty 1996; Rowe and Houle 1996).

For the genetic benefits to arise there must be additive genetic variation (V_A) for sexual ornaments, such that highly ornamented males can pass fitter genes to the progeny of choosy females. However, sexual selection is expected to erode V_A, leading to instability of female preferences (Pomiankowski et al. 1991; Pomiankowski and Iwasa 1998). Several mechanisms have been proposed to maintain V_A in sexual ornaments (reviewed in Radwan 2008). Among them, the genic capture hypothesis, stating that V_A in ornaments is maintained by pleiotropic effects of polymorphic genes affecting resource acquisition and use (Andersson 1994; Rowe and Houle 1996), has received most attention in the last decade (reviewed in Tomkins et al. 2004). The hypothesis implies that due to the costs of sexual ornaments, their expression is likely to depend on condition, defined as a pool of resources available for competing life-history traits (Rowe and Houle 1996). As the ability to acquire and handle resources is likely to be affected by a large number of genes, ornaments should thus "capture" variation in numerous loci underlying condition (Rowe and Houle 1996).

Traits affected by many genes are large targets for mutations, and mutational variance is a good predictor of standing genetic variance (Houle 1998). Thus, condition-dependent sexual traits can be expected to be sensitive to the load of deleterious mutations carried by a male. Indeed, Houle and Kondrashov (2002) showed that under a realistic rate of mutations affecting condition, female preferences for condition-dependent ornaments can easily evolve even if mate choice is costly. One of the consequences of the accumulation of detrimental mutations in the genome is a decrease in fitness-related traits under inbreed-

ing, called inbreeding depression. It is mainly attributed to recessive and partially recessive detrimental alleles, brought to the homozygous state by inbreeding (Roff 1997; Charlesworth and Charlesworth 1999). Traits under strong directional selection should show a high degree of directional dominance because dominant mutations that decrease trait value will be rapidly eliminated (Fisher 1930). Consequently, traits under strong directional selection should also suffer strong inbreeding depression (Charlesworth and Charlesworth 1999).

Indeed, fitness-associated life history traits do show higher inbreeding depression than morphological traits (DeRose and Roff 1999), which are more likely to be subjects of stabilising, rather than directional, selection. If sexually selected ornaments reflect condition *sensu* Rowe and Houle (1996), they should also exhibit higher inbreeding depression than typical morphological traits. However, this hypothesis has only been tested in a few cases so far, and the results are not consistent. In guppies, inbreeding decreases sexual coloration (Sheridan and Pomiankowski 1997; van Oosterhout et al. 2003) and intensity of courtship behavior (van Oosterhout et al. 2003; Mariette et al. 2006). In song sparrows, Reid et al. (2005) found strong inbreeding depression in male song repertoire size. Similarly, Aspi (2000) found a decrease in the frequency of male courtship song in *Drosophila montana*. However, Drayton et al. (2007) found no evidence for inbreeding depression in sexually selected calling effort in the cricket *Teleogryllus commodus* (although they found significant changes in three of five finer-scale call parameters), and Frommen et al. (2008) did not detect a significant inbreeding depression in breeding coloration in sticklebacks.

Here, we investigated whether male eyespan (the distance between the eyes), a sexually selected trait, shows increased sensitivity to inbreeding compared to other morphological traits in the stalk-eyed fly, *Teleopsis dalmanni*. Both males and females of stalk-eyed flies are characterized by eyes located on thin lateral extensions of the head capsule (eyestalks). In *T. dalmanni,* eyespan is highly exaggerated in males compared to females of similar body size (Baker and Wilkinson 2001), and females prefer to mate with males bearing larger eyespan (Wilkinson and Reillo 1994). Consistently with the genic capture hypothesis (Rowe and Houle1996), male eyespan is significantly more sensitive to environmental manipulations of condition than homologous female trait and other non-sexual traits (David et al. 1998; David et al. 2000; Cotton et al. 2004). To test whether it is also disproportionately affected by inbreeding, we investigated the effect of brother × sister mating on eyespan and two other morphological traits – thorax length and wing length – in both males and females.

Methods

Stock population

Flies used in this study were from a laboratory population derived from individuals collected in Malaysia by S. Cotton in 2005. Stock populations have since been maintained at high

density in 20×20×40cm cages and fed ground corn twice a week. Flies were kept at a constant temperature of 25°C with 12h:12h light : dark regime and 15 minute artificial dawn and dusk.

Experimental design

Virgin male and female flies were kept in separate cages until sexual maturity. After reaching maturity, they were randomly paired and each such established family was kept in a 1 litre plastic jar lined with moist cotton pads. Male and female offspring of each family were separated upon eclosion. After they reached sexual maturity, one male offspring of each family was mated to his sister and one was mated to a female offspring of another family, in order to create inbred and outbred pairs, respectively (Fig.1).

Inbred and outbred pairs were kept in 1 litre plastic jars lined with moist cotton pads. Feeding and egg collecting were performed three times a week. Collected eggs were placed in Petri dishes lined with moist cotton pads, with ground corn as food source for developing larvae. After eclosion, the progeny of all inbred and outbred pairs were frozen. Four randomly selected sons and daughters of each pair were photographed and their eyespan, thorax length and wing length were measured with the AnalySIS® image processing software (Soft Imaging System). Trait values were then averaged separately for male and female progeny of each pair.

Statistical analysis

Repeatability of measurement for all traits was assessed by photographing 42 flies (22 males and 20 females, randomly chosen from the stock colony) twice and each time scoring for eyespan, thorax, and wing size. Repeatability was then calculated according to Lessels and Boag (1987), as $\tau = s^2_A / (s_W^2 + s^2_A)$, where s^2_A is the among-groups variance component and s_W^2 is the within-group variance component, obtained from the analysis of variance with individual as independent variable. Correlations between traits (Pearson r) were also calculated using the same data.

Experimental data were first analysed with three-way ANOVA separately for each trait (eyespan, thorax and wing), with inbreeding treatment (inbred vs. outbred) and sex as fixed factors, plus family as a random factor.

To test whether inbreeding affects male eyespan after correcting for body size, we included thorax length (an index of body length) as an additional explaining variable in the model for eyespan. Male and female *T. dalmanni* have different eyespan-thorax allometries (see the significant effect of sex×thorax interaction on eyespan in Tab. 2), therefore, we ran a separate ANCOVA for each sex, with eyespan as a dependent variable, inbreeding and family as factors, and thorax length as a covariate. According to the predictions of the genic capture hypothesis, we expected a significant effect of inbreeding on male eyespan, but not on female eyespan.

As opposed to the progeny of inbred pairs, offspring of each outbred pair could be assigned either to their mother's or father's family (see Fig.1). With this pairing design it was

not possible to estimate the models with both the maternal and paternal families included simultaneously, because these factors would partition the same source of variation. Therefore, we ran two versions of each analysis, one with maternal family, and the other with paternal family as a random factor. Since the results from these alternative analyses were qualitatively the same, indicating that the statistical difficulty related to the breeding design did not affect the main conclusions, only the results of the analyses using paternal families are shown below.

The analyses were performed in Statistica 8.0 (StatSoft®).

For comparative purposes, we calculated the slope of change in trait values as a result of inbreeding (assuming linear relationship between trait value and the inbreeding coefficient F) standardised by the outbred trait mean: $b_{Xo} = (X_O - X_I)/FX_O$ (De Rose and Roff 1999), where X_O is the mean trait value in outbreds, X_I is the mean trait value in inbreds, and F is Wright's (1921) inbreeding coefficient (0.25 for brother-sister mating applied in our study).

Results

All morphological traits we measured showed significant repeatability (males: thorax $\tau = 0.48$, $p < 0.01$; wing $\tau = 0.86$, $p < 0.001$; eyespan $\tau = 0.96$, $p < 0.001$; females: thorax $\tau = 0.78$, $p = 0.001$; wing $\tau = 0.82$, $p < 0.001$; eyespan $\tau = 0.88$, $p < 0.001$) and were significantly correlated with each other (Pearson correlation coefficients for females: thorax vs. eyespan $r = 0.57$, wing vs. eyespan $r = 0.64$, wing vs. thorax $r = 0.57$; for males: thorax vs. eyespan $r = 0.63$, wing vs. eyespan $r = 0.60$, wing vs. thorax $r = 0.59$; $p < 0.01$ for all correlations).

Mean values for inbreds and outbreds of both sexes are shown in Fig. 2 a,b,c. All traits showed significant inbreeding depression (Table 1).

Male eyespan decreased with inbreeding more than female eyespan, as indicated by the significant effect of inbreeding×sex interaction on this trait (Table 1). However, larger decline in the absolute value of eyespan could result simply from the fact that in *T. dalmanni* eyespan is far larger in males than in females (Baker and Wilkinson 2001). In order to check whether proportional decline in eyespan under inbreeding was also larger in males, we compared inbreeding coefficients (b_{Xo}, calculated separately for males and females from each family) between sexes with Student's t test. We found that proportional decline did not differ between males and females ($t = 0.55$; $p = 0.59$).

The inbreeding depression coefficient (b_{Xo}) for male eyespan was 0.13, which overlapped the range of values observed for other morphological traits (Fig. 2 a,b,c).

After accounting for thorax length, the inbreeding effect was no longer significant either on female ($F = 0.0007$, $p = 0.98$), or on male eyespan (Tab. 3, see also Fig. 3).

Figures and Tables

Figure 1. Crossing scheme used to generate inbred and outbred progeny, on which the measurements were performed

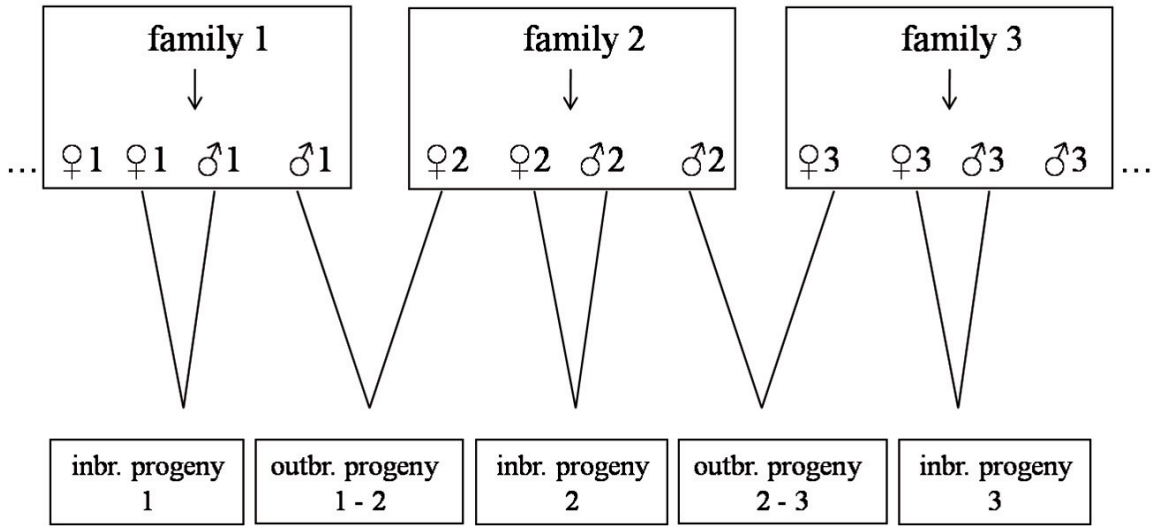

Figure 2. Means (± SE) of morphological traits in males and females from inbred (filled symbols) and outbred (open symbols) families (four males and four females per family measured). Coefficient b_{Xo} indicates the slope of change of trait value as a result of inbreeding, standardized by the outbred trait mean.

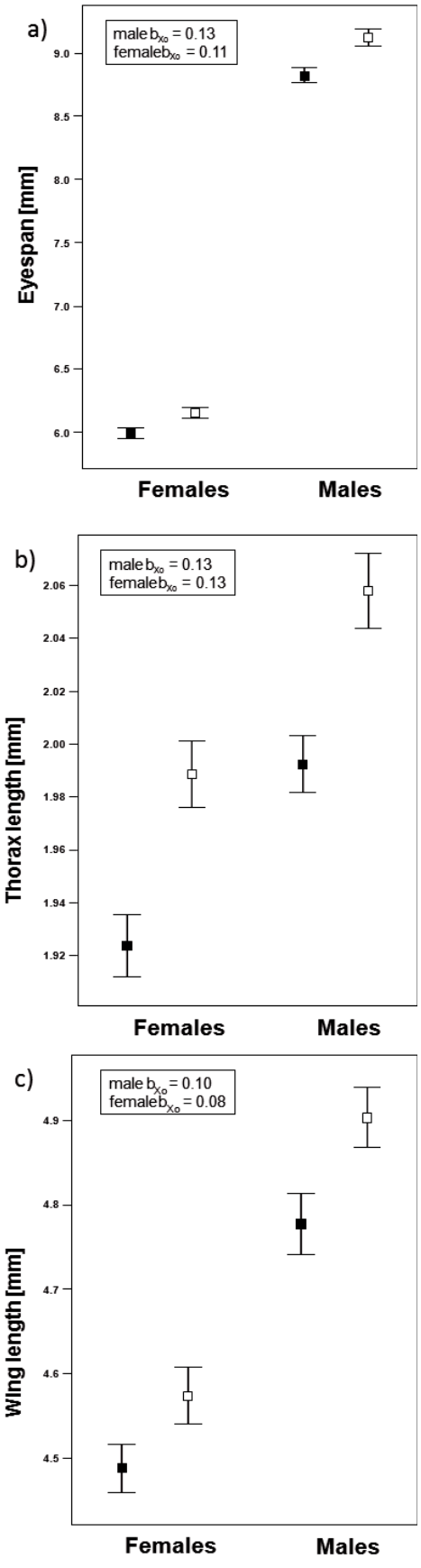

Figure 3. Eyespan to thorax relationship in outbred and inbred males.

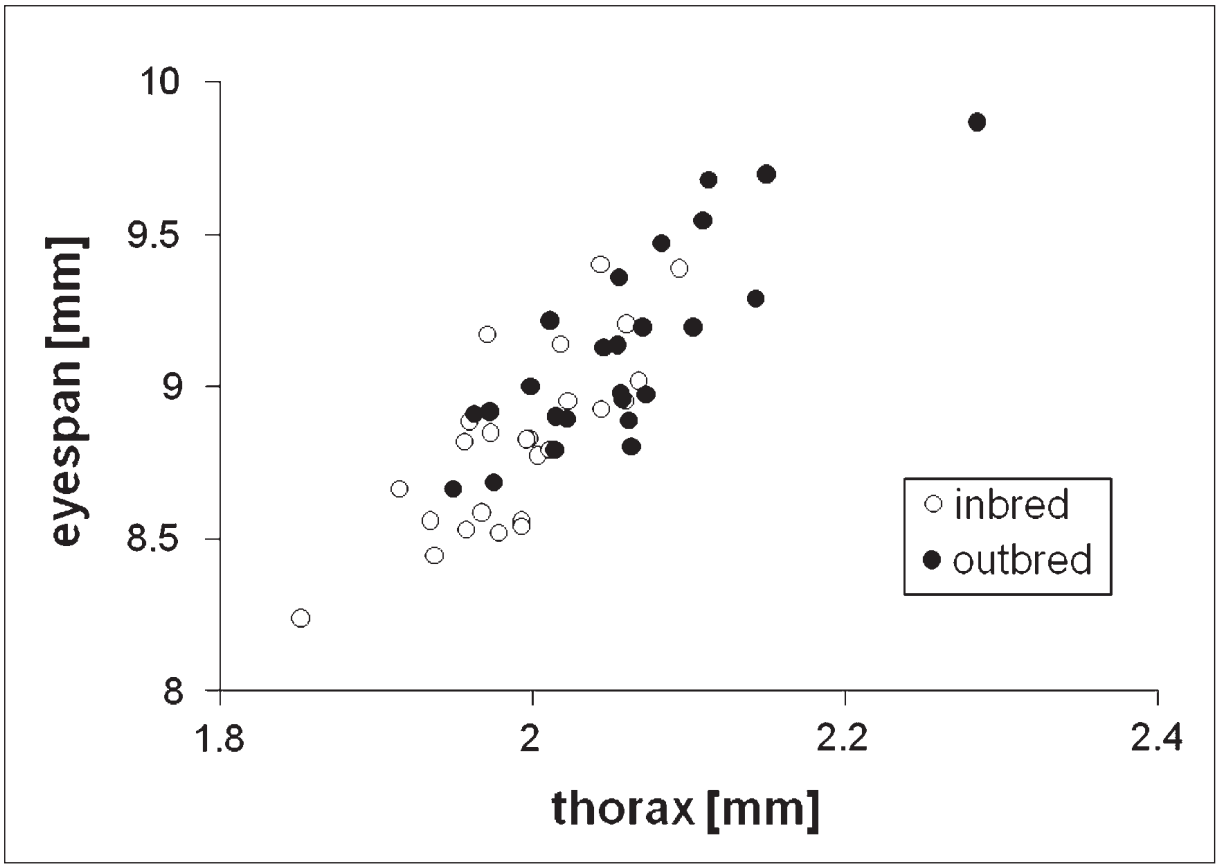

Table 1. Results of GLM testing the effects of inbreeding (inbr.), sex, father's family and thorax length (covariate) on eyespan (analyses using maternal, instead of paternal, family gave qualitatively identical results). Interactions with family were not included in the model, and all non-significant interactions with covariate were removed.

Effect	*df*	MS	*F*	*P*
Inbr.	1	679	2.6	0.113
Sex	1	25	0.1	0.759
Father	24	558	2.1	0.008
Thorax	1	24,447	92.8	<0.001
Inbr. × sex	1	230	0.9	0.353
Sex × thorax	1	1,738	6.6	0.012
Error	70	264		

Table 2. Results of ANOVA testing the effects of inbreeding (inbr.), sex, and father's family on eyespan, thorax and wing (analyses using maternal, instead of paternal, family gave qualitatively identical results). The three-way interaction was not included in the model and sex ×

Effect	*df*	Eyespan			Thorax			Wing		
		MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Inbr.	1	18,591	13.8	0.001	1,458	14.5	<0.001	3,835	5.9	0.023
Sex	1	287,847	10,665.5	<0.001	1,622	93.4	<0.001	32,656	371.1	<0.001
Family	24	2,010	1.5	0.167	75	0.7	0.757	708	1.1	0.425
Inbr. × sex	1	1,673	6.2	0.016	0	0.0	0.977	138	1.6	0.217
Inbr. × family	24	1,349	5.0	<0.001	101	5.8	<0.001	655	7.4	<0.001
Error	48	270			17			88		

Table 3. Results of ANCOVA testing the effects of inbreeding (inbr.), father's family and thorax length (covariate) on male eyespan (analyses using maternal, instead of paternal, family gave qualitatively identical results). Interactions with family were not included in the model.

Effect	*df*	MS	*F*	*P*
Inbr.	1	865	2.0	0.168
Father	24	554	1.3	0.268
Thorax	1	16,981	39.7	<0.001
Error	23	427		

Table 4. Literature data on inbreeding depression in sexually selected traits. The inbreeding coefficient F (Wright 1921) was calculated from the number of generations of experimentally applied inbreeding in all studies except that by Reid and co-workers, who calculated it using the molecular markers data. The b_{Xo} coefficients were calculated based on data shown in the papers. Italicized trait names indicate that the effect of inbreeding was statistically significant.

Model	Trait	F	b_{Xo}	Reference
Guppy, Paria population	*Relative orange spot area*	0.25	0.558	Sheridan and Pomiankowski (1997)
Guppy	Relative orange spot area	0.25	0.815	Mariette et al. (2006)
Guppy	*Sigmoid displays*	0.25	2.750	Mariette et al. (2006)
Guppy	*Female following behaviour*	0.25	1.385	Mariette et al. (2006)
Song sparrow	*Song repertoire size*	0.00–0.18	3.5 2.0	Reid et al. (2005)
Drosophila montana	*Song frequency*	0.986	0.086–0.128	Aspi (2000)
Threespine stickleback	Red throat coloration	0.25	- 0.07 - 0.06	Frommen et al. (2008)
Threespine stickleback	Blue eye coloration	0.25	- 0.07 0.00	Frommen et al. (2008)
Field cricket	Calling effort	0.25	- 0.199	Drayton et al. (2007)

Discussion

All traits we measured showed small, but significant decline under inbreeding. DeRose and Roff (1999) found that the average slope of decline in body size as a result of inbreeding, b_{Xo}, was 0.230 (SE=0.12, n=15 species, median=0.086). Our estimates are close to the median value shown by these authors, indicating that inbreeding depression in morphological traits in *T. dalmanni* is well within the range reported for other species.

The genic capture hypothesis predicts that, similarly to life-history traits, sexual ornaments should capture genetic variation in traits affecting resource acquisition and use (Rowe and Houle 1996). Therefore, ornaments should resemble life-history traits in being subject to strong directional selection (Pomiankowski and Moller 1995), which gives rise to strong directional dominance (Lynch and Walsh 1998). Consequently, sexually selected ornaments should show higher inbreeding depression than non-sexual morphological traits, including homologous female traits if present.

In our study, male eyespan decreased under inbreeding more than female eyespan. However, this was only true for decline in absolute trait value; proportional decline did not differ between sexes. Moreover, after including thorax length (an index of body length) in the model for male eyespan, the effect of inbreeding was no longer significant, showing that male eyespan's decline under inbreeding can be explained by overall decline in body size.

The magnitude of inbreeding depression in male eyespan (b_{Xo} = 0.13) was much lower than the average b_{Xo} for life-history traits reported by DeRose and Roff (n=52 species, mean ± SE =1.600 ± 0.37, median=0.582). Even though we did not, for logistic reasons, measure life history traits in this experiment, the comparison with data presented in DeRose and Roff (1999) indicates that male eyespan in *T. dalmanni* does not show the level of directional dominance characteristic of life-history traits. In fact, b_{Xo} for male eyespan fits well in the range reported for morphological traits (see above). Thus, a considerable proportion of high genetic variation for male eyespan reported for this species (Wilkinson and Taper 1999) may be due to sources other than the variance in condition *sensu* Rowe and Houle (1996).

Indeed, Johns et al. (2005) have recently found four QTLs for male eyespan, explaining jointly 53% of the variance in this trait, with a single QTL on the X chromosome explaining as much as 36%. This indicates that eyespan may be determined by a small number of loci of large effect, rather than by a large number of loci affecting condition, as predicted by the genic capture hypothesis.

An alternative explanation for our findings is that effects of inbreeding on male ornament might have been obscured by mild environmental conditions experienced by the flies during our study. In *T. dalmanni*, male eyespan is more sensitive to low food availability during larval period than non-sexual morphological traits (Cotton et al. 2004). Differences in eyespan between genotypes are also more pronounced when larvae are raised in harsher environment (David et al. 2000). Indeed, if male ornaments capture genetic variation in condition

(Tomkins et al. 2004), then ornaments of low genetic quality individuals should be impaired more severely in harsh environments than in conditions where resources are readily available. In our experiment, larvae had *ad libitum* access to high quality food, which might have partially masked the effects of inbreeding on male eyespan.

However, Armbruster and Reed (2005) found that although inbreeding depression in fitness-related traits does usually increase in stressful environmental conditions, this pattern is far from universal. In fact, in 24% of the cases included in their meta-analysis, the opposite results were found. Moreover, there is evidence that life history traits do show substantially higher inbreeding depression than morphological traits even in mild environmental conditions (Wright et al. 2008; Michalczyk 2008). Similar difference would be expected between ornaments and morphological traits if the former resembled life histories in terms of condition dependence and directional dominance, as predicted by the genic capture hypothesis. Indeed, several studies did find severe inbreeding depression in sexual traits without manipulating environmental conditions (see discussion below).

It is worth noting here that environmental and genetic manipulations of condition may sometimes have inconsistent effects on male ornaments. For example, in field crickets, inbreeding does not reduce male call effort (Drayton et al. 2007), a sexually selected trait that is sensitive to food quality (Hunt et al. 2004). Such pattern, similar to our finding of relatively low inbreeding depression in a sexual trait known to be sensitive to environmental manipulation (Cotton et al. 2004) may reflect relatively low priority of resource allocation in sexual ornament, compared with traits that are more essential for body maintenance, and hence survival and reproduction (Glazier 2002). Conversely in guppies, where orange spot patterns show significant inbreeding depression (Sheridan and Pomiankowski 1997; van Oosterhout et al. 2003), food availability affects body mass, but not area of orange spots, even though coefficients of genetic variation in orange spot area exceed that of male size by an order of magnitude (Hughes et al. 2005).

Consequently, experiments showing sensitivity of sexual traits to environmental manipulation of condition, interpreted as a support of the genic capture mechanism (e.g. Tomkins et al. 2004), may in fact carry little information about the impact of genetic quality on the expression of sexual ornaments.

Sensitivity of sexually selected ornaments to inbreeding thus appears to vary (Table 3). In some species (such as guppies and song sparrows) sexual traits show inbreeding depression of the level characteristic for life history traits; in others (field crickets, threespine sticklebacks) they show no indication of inbreeding depression whatsoever. In others still, ornaments decline significantly with inbreeding, but the magnitude of this decline is comparable with morphological rather than life history traits – this is the case with *Drosophila montana* (Table 3), Alpine ibex (von Hardenberg et al. 2007), and *T. dalmanni* studied in this paper.

These differences in sensitivity of sexual ornaments to inbreeding remain to be explained. One possibility is that they may result from different mechanisms of sexual selection

operating in different species. Our study shows that in *T. dalmanni,* inbreeding depression in male ornament (eyespan), although highly significant statistically, is within the range typical of morphological rather than life history traits, which does not provide strong support the genic capture version of the 'good genes' hypothesis. However, it is possible that other 'good genes' mechanisms, such as avoiding sex-ratio distorters (Wilkinson et al. 1998), may be responsible for maintaining female preferences for males with large eyespan.

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Chapter 2.

Does sexual ornament size signal mutation load in a stalk-eyed fly *Teleopsis dalmanni*?

Introduction

Evolution of male sexual ornaments (epigamic traits) and female preferences for them is one of the core unresolved issues in the area of sexual selection. Zahavi (1975, 1977) proposed that ornaments may reflect male quality, hence choosing more ornamented mating partners may benefit females by ensuring higher genetic quality of their offspring. However, strong directional selection, resulting from female preferences and leading to tight correlation between male reproductive success and the level of ornamentation, is expected to deplete genetic variation in both ornaments and "good genes" underlying their expression. This so called lek paradox (e.g. Taylor and Williams 1982) was theoretically solved by a genic capture hypothesis, linking the expression of epigamic traits to male physiological condition, defined as a pool of resources available for competing life-history traits (Andersson 1986; Rowe and Houle 1996). Since virtually every locus in the genome will likely influence the efficiency of resource acquisition and processing in some way, condition is expected to depend on a huge number of genetic loci, therefore providing a large target for mutations (Houle 1998), see *Discussion*. Houle and Kondrashow (2002) showed that the evolution of costly ornaments may proceed if their expression is condition-dependent and *de novo* deleterious mutations maintain genetic variation in condition. However, studies relating the expression of male ornamental traits to mutation load are almost nonexistent.

Several papers analysed the efficiency of sexual selection in purging deleterious mutations from populations. Radwan (2004) exposed bulb mite populations to mutagenic ionising radiation and recorded a resulting decline in viability. The mutated populations were then subjected to one generation of either fairly strong or no sexual selection. Embryo viability was measured in a subsequent generation, and was shown to be over 80% higher in the populations that passed through the sexual selection treatment. Hollis et al. (2009) tracked the frequency of a deleterious Adh^{-} allele in *Drosophila melanogaster* populations that either experienced sexual selection or did not. They found that the allele was removed more rapidly from populations where sexual selection was allowed to operate. Pischedda and Chippindale (2005) found that males carrying a deleterious nub^{1} mutation had lower mating success than wild-type males. However, neither of these studies enables a clear distinction as to whether the observed effects of sexual selection on mutation load were due to deleterious mutations hampering male epigamic traits or rather the traits involved in male-male competition for mates.

Moller and Mousseau (2003) reported that in barn swallows, male epigamic traits were more affected by radioactive pollution than morphological traits unrelated to sexual selec-

tion, suggesting that sexually selected traits are more sensitive to mutations. However, these results come from a correlational, rather than a controlled experimental study and therefore they do not constitute a rigorous test of the genic capture hypothesis. Pekkala *et al.* (2009) found a significant but weak negative effect of induced mutations on male courtship behavior.

In this chapter, I attempted to test genic capture hypothesis using a stalk-eyed fly *Teleopsis dalmanni (Diopsidae).* Both male and female diopsids are characterized by eyes located on thin lateral extensions of the head (eye-stalks). In *T. dalmanni*, eyespan is highly exaggerated in males compared to females of similar body size and females prefer to mate with males bearing larger eyespans (Wilkinson and Reillo 1994; Hingle et al. 2001), which qualifies this trait as a sexual ornament. Male eyespan is significantly more sensitive to environmental manipulations of condition than homologous female trait and other non-sexual traits (David et al. 1998; David et al. 2000; Cotton et al. 2004). On the other hand, it does not show high inbreeding depression expected to characterise condition-dependent traits (Prokop et al. 2010).

Here, I applied ionising radiation to induce mutations, enabling direct comparison of ornament size between groups of flies differing in mutation load. Based on the genic capture hypothesis, I predicted that male eyespan should be more affected by the mutation load, and therefore decrease more steeply with increasing irradiation dose, than non-sexual morphological traits. I expected a decline in eyespan to be comparable to that in life history traits which are considered to be influenced by numerous loci (Houle 1998, Merila and Sheldon 1999).

Methods

Stock population

Flies used in this study were from a laboratory population derived from individuals collected in Malaysia by A. Pomiankowski in 1993. Stock populations have since been maintained at high density in 20 × 20 × 40 cm cages and fed twice a week with ground corn or a mixture of blended banana and yeast *ad libitum*. They were kept at a constant temperature of 25°C with a 12h:12h light:dark regime and 15 min artificial dawn and dusk.

General procedures

Ionising gamma rays were applied in order to manipulate the level of mutation load. Six doses of radiation (1, 2, 3, 4, 5 and 6 kRad) plus a non-irradiated control (0 kRad) were used. The resulting mutation load is expected to increase with irradiation dose (Evans & DeMarini 1999). For logistic reasons, the experiment was performed in two replicates, with 123 sexually mature males in the first and 166 in the second replicate. Males were randomly assigned to doses. Within several hours from irradiation, each male was paired with a non-irradiated virgin female to form an experimental P generation. Each pair was kept in a 1l plastic jar lined with moist cotton pads and tissue paper, and fed with a mixture of blended banana and yeast *ad libitum*. Every third day, lining and food were replaced, and old lining with eggs laid by a female

was transferred to a 100ml plastic container, where food *ad libitum* was provided to developing larvae. Progeny of each experimental pair was collected in this way for 3 weeks, unless a female died earlier. *Ca.* 8-10 days after egg collection, open containers with pupae were placed individually in 5l plastic cages and eclosing individuals (F1 generation) were counted, sexed, and raised in same-sex sib groups to maturity. Individuals that died before reaching maturity were immediately collected and preserved in -20°C. All individuals surviving to maturity were then used for life history assays.

In order to assess direct effects of gamma irradiation on male fertility, I recorded the numbers of fertile and infertile P generation pairs as well as the number of offspring produced by each of the fertile one.

Effects of inherited mutations on offspring traits

Morphological traits

Morphological traits were scored for all eclosed F1 males and females, apart from those that were physically damaged and therefore impossible to measure. Flies were either collected dead (see above) or killed by freezing, then dissected and photographed under a stereomicroscope. Eyespan, wing length and hind leg tibia length were measured with the AnalySIS® image processing software (Soft Imaging System). Male eyespan is a sexual ornament, whereas female eyespan and the other two traits in both sexes are considered non-sexual morphological traits.

Life history traits

Fecundity of all F1 females that survived to maturity was measured. Each female was paired with a young stock male in a 1l plastic jar lined with moist cotton pad (see *General procedures*). Every third day, lining and food were replaced, and eggs on the old lining were counted. This procedure was continued for 1 month unless a female died earlier (males were replaced if dead). The mean number of eggs per a 3-day period (averaged over the period of 1 month or less if a female died before completing the assay) was used as a fecundity measure of each female.

Fertility of all F1 males that survived to maturity was measured. Each male was placed in a 1 plastic jar (see *General procedures*) with two virgin stock females. Every third day, lining and food were replaced. Old lining was placed in a Petri dish and left for 4 days to ensure that all viable eggs have hatched. After 4 days, both the total number of eggs and the number of hatched eggs were scored. This procedure was continued for 1 month unless a male died earlier (females were replaced if dead or infertile). Male fertility was measured as a total number of hatched eggs summed over a 1 month period (or shorter if a male died before completing the assay). This measure obviously depends also on the total number of eggs laid by a male's

partners during that time, which was therefore included as a covariate in the model for male fertilit.

Statistical analyses

All statistical analyses were performed using R 2.12.1 (R Development Core Team, 2010).

Direct influence of irradiation on male fertility was assessed by analysing the effects of irradiation dose and experimental replicate on the probability of producing, and the number of, the F1 offspring. Probability of producing offspring was analysed using generalised linear model with the binomial error distribution and a logit link function. The number of offspring produced was analysed using generalised linear model with the quasipoisson error distribution (dispersion parameter 7.61) and a log link function; offspring sex was included as an additional predictor variable in this model.

In order to assess the influence of inherited mutations on the ornament and non-sexual morphological traits in individuals heterozygous for these mutations, I analysed the effects of irradiation dose, experimental replicate and sex on eyespan, wing length and tibia length of the F1 offspring. I used generalised linear mixed models with the normal error structure and the sire (P generation male) ID as a random factor. These models were fitted using the Markov Chain Monte Carlo method implemented in R package MCMCglmm (Hadfield 2010). Residual plots were used to verify that the assumption of a normal error distribution was appropriate and the *autocorr* function implemented in MCMCglmm package was used to check for autocorrelations.

To assess the influence of inherited mutations on life history traits in individuals heterozygous for these mutations, I analysed the effects of irradiation dose and experimental replicate on the fecundity of F1 females (dependent variable: mean number of eggs laid per 3 days) and fertility of F1 males (dependent variable: the number of hatched eggs; total number of eggs included as an additional covariate in the model). I used generalised linear mixed models with the normal error structure and the sire (P generation male) ID as a random factor. The models were fitted and verified in the same way as those for morphological traits.

In all the above models, irradiation dose was treated as a continuous fixed variable, and experimental replicate as a fixed factor. The reason for the latter was that there was an evident difference between the replicates in the mortality of experimental (P generation) pairs, suggesting a decline in the quality of laboratory conditions in the second replicate.

Numbers of offspring and sires included in each of the models analysing progeny traits are given in Table 1.

Results

Gamma rays dose had a strong negative effect on the fertility of irradiated males: both the proportion of fertile males and the number of F1 offspring produced decreased significantly with the increasing dose (Tables 2-3).

There was no effect of irradiation on any of the offspring morphological traits measured (Table 4). I had predicted that heightened sensitivity of male ornament to mutations should be manifested by a significant effect of sex × irradiation dose interaction on eyespan, with male eyespan declining more with the increasing dose than female eyespan. However, I found no such effect (Table 5 and Fig. 1).

Fertility of sons tended to decrease with the increasing irradiation dose, although this effect was marginally non-significant (Fig. 2, Table 6). Fecundity of daughters also tended to decline with the increasing dose but this effect was not significant as well (Table 7).

Figures and Tables

Figure 1. Effect of irradiation dose received by sires (P generation males) on F1 male and female eyespan. Mean values for sons and daughters of each irradiated (P generation) male are shown.

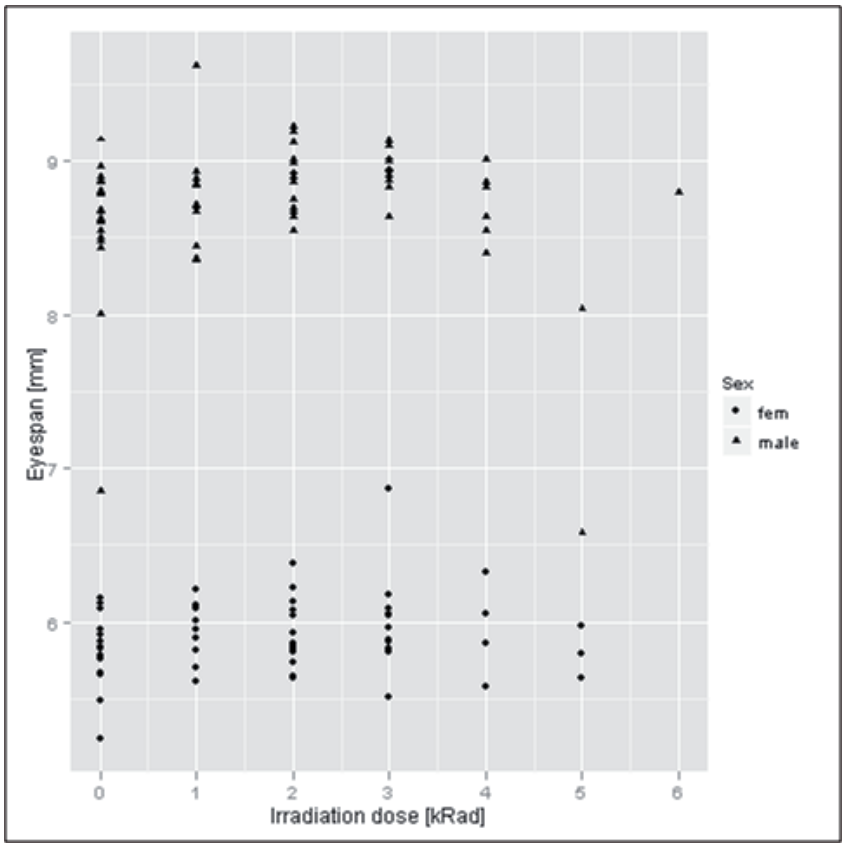

Figure 2. Effect of irradiation dose received by sires (P generation males) on fertility of their sons. Mean values of residual offspring number with respect to the total number of eggs laid are shown for sons of each irradiated male.

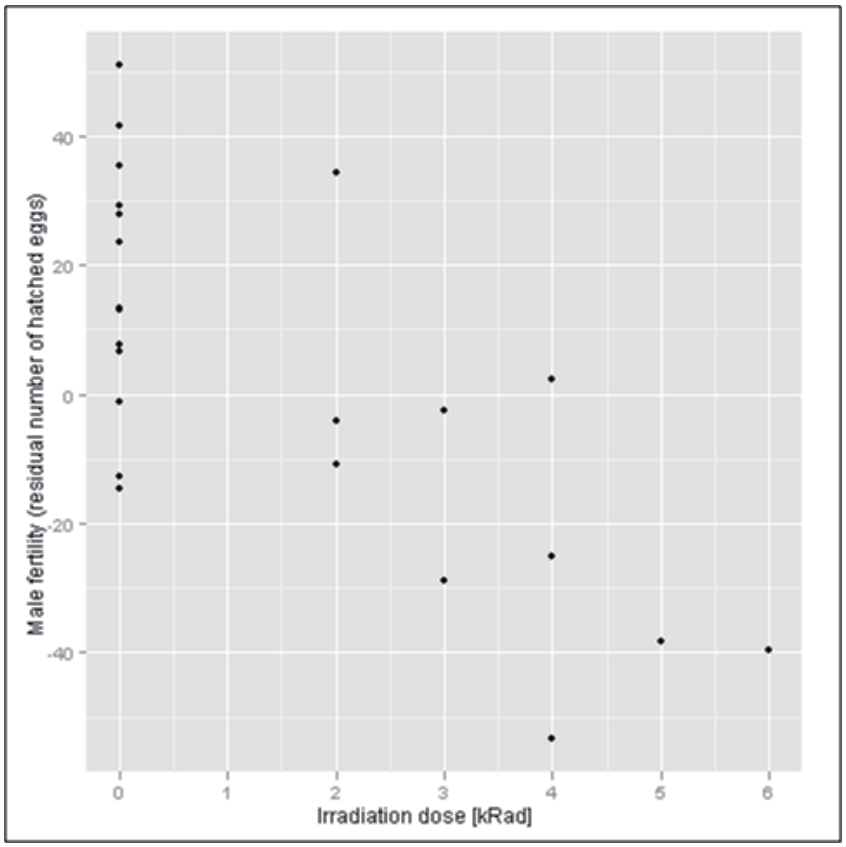

Table 1. Numbers of sires and offspring included in the analyses of offspring morphological and life history traits

	eyespan		wing length		tibia length		female fecundity		male fertility	
Irradiation dose	sires number	offspring number	sires number	offspring number	sires number	offspring number	sires number	offspring number	sires number	offspring number
0 kRad	21	148	21	95	21	138	19	63	13	23
1 kRad	13	35	11	24	13	34	8	11	0	0
2 kRad	18	69	17	51	15	60	9	28	3	16
3 kRad	15	42	13	33	14	38	2	3	2	5
4 kRad	11	20	8	14	10	19	2	2	3	4
5 kRad	4	5	2	3	4	4	1	2	1	1
6 kRad	1	1	1	1	1	1	3	3	1	1

Table 2. Results of a generalized linear model (binomial error distribution, logit link function) testing the effect of irradiation dose on the probability of producing offspring by experimental (P generation) males.

Effect	Estimate	SE	z	P
Intercept	1.10	0.31	3.58	0.000
Experimental replicate (2^{nd})	-2.17	0.31	-7.06	0.000
Irradiation dose	-0.32	0.08	-3.92	0.000
Residual deviance:		292.32 on 285 degrees of freedom		

Table 3. Results of a generalized linear model (quasipoisson error distribution, log link function) testing the effect of irradiation dose on the number of offspring produced by experimental (P generation) males.

Effect	Estimate	SE	t	P
Intercept	2.67	0.16	17.16	0.000
Experimental replicate (2^{nd})	-0.82	0.27	-3.07	0.003
Sex (Male)	-0.03	0.18	-0.18	0.855
Irradiation dose	-0.48	0.08	-6.25	0.000
Residual deviance:		715.4 on 139 degrees of freedom		

Table 4. Slope estimates for irradiation dose effects on offspring morphological traits, obtained from generalized linear mixed models (random factor: sire ID). Each model also included an experimental replicate (not significant in any model) and offspring sex (males larger than females in all traits) as fixed factors (results not shown).

Trait	Posterior mean (irradiation dose slope)	Confidence interval	pMCMC
Eyespan	0.02	-0.03 – 0.07	0.394
Wing	0.01	0.00 – 0.02	0.223
Tibia	0.01	0.00 – 0.02	0.220
Note: numbers of individuals measured are given in Table 1.			

Table 5. Results of the generalized linear model (random factor: sire ID) for offspring eyespan. Sex × dose interaction tests the hypothesis of heightened sensitivity of male ornament (eyespan) to mutations.

Effect	Posterior mean	Confidence interval			pMCMC
Intercept	5.77	5.50	-	6.06	0.000
Sex (Male)	3.39	3.09	-	3.68	0.000
Experimental replicate (2^{nd})	0.07	-0.14	-	0.28	0.533
Irradiation dose	0.03	-0.02	-	0.10	0.261
Sex (Male) × Experimental replicate (2^{nd})	-0.44	-0.68	-	-0.19	0.000
Sex (Male) × Irradiation dose	-0.03	-0.09	-	0.03	0.376

Note: numbers of individuals measured are given in Table 1.

Table 6. Results of the generalized linear model (random factor: sire ID) testing the effect of irradiation dose received by sires (P generation males) on their sons' fertility.

Effect	Posterior mean	Confidence interval			pMCMC
Intercept	-3.42	-24.76	-	15.49	0.726
Irradiation dose	-6.16	-12.52	-	-0.17	0.056
Number of eggs laid	0.92	0.79	-	1.03	0.000

Note: numbers of individuals measured are given in Table 1.

Table 7. Results of the generalized linear model (random factor: sire ID) testing the effect of irradiation dose received by sires (P generation males) on their daughters' fecundity.

Effect	Posterior mean	Confidence interval			pMCMC
Intercept	7.61	6.25	-	8.87	0.000
Experimental replicate (2^{nd})	-2.37	-4.40	-	-0.35	0.019
Irradiation dose	-0.56	-1.26	-	0.20	0.128

Note: numbers of individuals measured are given in Table 1.

Discussion

As indicated by numerous mutation accumulation experiments, the net effect of *de novo* mutations is deleterious (reviewed by Halligan and Keightley 2009). However, this effect differs considerably between species and traits (Halligan and Keightley 2009). Traits that are controlled by numerous genetic loci are expected to be particularly sensitive to mutations (Houle 1991) because they present large 'targets,' *i.e.* cover a substantial proportion of a genome and therefore are likely to be 'hit' by *de novo* arising mutations. This prediction is supported by the positive correlation between the magnitude of mutational variance and inferred target size of traits in *Drosophila melanogaster* (Houle 1998) and by the fact that life history traits, which are likely affected by numerous loci, tend to show higher sensitivity to mutations compared to morphological traits which are considered to have less complex genetic architecture (Houle et al. 1996; Houle 1998; Merila and Sheldon 1999) but see Tomkins et al (2009). Genic capture hypothesis predicts that sexual ornaments are dependent on physiological condition, and therefore indirectly affected by a large number of genes underlying condition (Rowe and Houle 1996). Thus, they should be highly sensitive to mutations.

In this study, ionising radiation was used to induce mutations in male stalk-eyed flies and the effect of inherited heterozygous mutations on sexual ornament (eyespan) of their sons was analysed. As a reference, non-sexual morphological traits (wing and tibia lengths in both sexes and female eyespan) and life history traits (male and female fertility) were also measured in the progeny of irradiated males. Based on genic capture hypothesis (Rowe and Houle 1996), I expected the decrease in male ornament to be similar to that in the life history, rather than other morphological, traits.

In accordance with the mutational target hypothesis (Houle 1991, 1998), both male and female fertility tended to decrease with increasing irradiation dose (although these effects did not reach statistical significance), whereas no such effect was observed for morphological traits. Contrary to the predictions of the genic capture hypothesis, there was no indication of a decline in male ornament with mutation load: male eyespan did not differ in that respect from female eyespan or other non-sexual morphological traits. This finding is in accord with the results obtained by Prokop et al. (2010) (chapter 1 of this thesis) who found that the magnitude of inbreeding depression in male *T. dalmanni* eyespan is relatively low, fitting in the range reported for morphological rather than life history traits (DeRose and Roff 1999).

Therefore, high genetic variation for male eyespan reported for this species by Wilkinson and Taper (1999) seems to be due to sources other than the variation in condition *sensu* Rowe and Houle (1996). Indeed, Johns *et al.* (2005) found four QTL for male eyespan in *T. dalmanni*, explaining jointly 53% of variance in this trait, with a single QTL on the X chromosome explaining as much as 36%. This indicates that male eyespan may be determined by several genomic regions (loci or sets of linked loci) of large effects, rather than by a large number of

loci scattered throughout the genome and affecting condition, as predicted by the genic capture hypothesis.

Data on the influence of mutations on male ornaments in other systems is surprisingly scarce. Moller and Mousseau (2003) compared a range of ornamental and non-sexual morphological traits in populations of barn swallows originating from two geographic regions differing in the level of radioactive pollution. They found that male ornamental traits were more affected by radioactive pollution than non-sexual, suggesting that sexually selected traits are more sensitive to mutations. However, their research approach does not enable unequivocal conclusions regarding the genic capture hypothesis for two reasons. First, factors other than radioactivity level, distinguishing the two regions but unaccounted for by the authors, may have influenced the observed effects. Second, even if the effects indeed resulted from the mutations caused by radioactivity, it is unclear whether those mutations affected the germline or somatic tissues only. This is a crucial issue, since ornament expression unrelated to genetic load in the germline will not provide any indicator of the number of deleterious mutations a male's progeny will inherit. To my knowledge, the only work directly studying the impact of heritable deleterious mutations on male display traits was published by Pekkala et al. (2009). The authors manipulated the mutation load in *Drosophila montana* males by inducing mutations with different doses of ionising radiation, and assessed the effects of inherited heterozygous mutations on courtship activity. They found that mutations reduced the probability of, and extended the latency to, male courtship in one male – one female mating trials, yet these effects were relatively small and no influence on the actual mating success was observed, providing a weak, if any, support for genic capture. However, Pekkala and colleagues did not compare the effects of mutations on courtship behavior with the effects on other types of characters, particularly life history traits. Therefore, it cannot be excluded that their results are due to a generally low influence of irradiation on the number of mutations in sperm cells (which could result from effective mechanisms guarding the genetic integrity of the germline) or conversely, strong selection at the embryonic and/or larval stage eliminating mutants and retaining only those individuals that were relatively mutation-free, even in high dose treatments.

The generality of my finding of no support for genic capture hypothesis therefore remains to be established. In *Teleopsis dalmanni*, other 'good genes' mechanisms such as avoiding sex-ratio distorters (Wilkinson et al. 1998), or direct fertility benefits (Cotton et al. 2010, Rogers et al. 2008) may be responsible for maintaining female preferences for large-eyespan males.

Chapter 3.

Male attractiveness and offspring fitness – a meta-analysis

Introduction

Female preferences for specific male phenotypes have been documented for a wide range of taxa (reviewed in Andersson 1994). The evolutionary mechanisms responsible for the origin and maintenance of such preferences, especially in non-resource-based mating systems in which males contribute only gametes to the next generation, still remain to be satisfactorily explained (reviewed by Kokko et al. 2003, Kokko et al. 2006). One of the commonly invoked mechanisms is indirect selection, which occurs when preference alleles are in linkage disequilibrium with other alleles that are selected for (Kirkpatrick and Barton 1997).

Fisher (1930) proposed that genes for female preferences can spread in populations because they are passed onto sons who also inherit the genes for sexually attractive trait(s) from their fathers, thereby creating a linkage disequilibrium between preference and preferred trait. This leads to a positive feedback loop: female choice selects for male attractiveness trait(s), causing a correlated selection on choice itself. Another line of explanation was offered by the handicap principle (more commonly discussed under a later coined name of the good genes hypothesis), proposed by Zahavi (1975): female preferences evolve and are maintained because male ornaments signal genetic quality, or more precisely, breeding value for fitness (Tomkins et al. 2004, Hunt et al. 2004). The crucial prediction of this hypothesis is a positive genetic correlation between male attractiveness and total fitness (Kirkpatrick and Barton 1997).

To date, numerous studies have been carried out on a wide range of animal taxa, with the aim of testing this prediction. Since fitness is notoriously difficult to measure, various fitness-related traits are measured instead in such studies. Moller and Alatalo (1999) published a meta-analysis of 22 papers reporting on the relationships between father attractiveness traits and offspring survival. They found a week, yet significant, positive relationship with the average correlation coefficient of 0.122 (Moller and Alatalo 1999). However, survival is only one of the many fitness components, likely traded against other life-history traits (e.g. Bochdanovits and de Jong 2004). Therefore, the relationship between male epigamic traits and offspring survival alone tells us little about the relationship between those traits and offspring fitness, and thus about the “good genes” consequences of female choice. Inclusion of other fitness-related traits into a meta-analysis would give a much more comprehensive view of the importance of good genes effects. In fact, definition of good genes should comprise all alleles affecting fitness, including those contributing to reproductive success via sexual attractiveness. This blurs the long-standing distinction between “Fisherian” and “good genes” hypotheses, which can more appropriately be viewed as two extremes of a continuum (eg. Kokko et al. 2002, 2006).

An important confounding factor, often associated with studies of sire attractiveness – offspring fitness correlations, is differential maternal allocation, *i.e.* correlation between female reproductive investment and her mate attractiveness (Sheldon 2000). This phenomenon has been reported from many taxa and, if not controlled for in the studies of good genes effects, may bias the estimates of sire attractiveness – offspring fitness correlations.

Dozens of studies have been published since the meta-analysis of Moller and Alatalo (1999), some of which also controlled for differential maternal allocation. In this chapter, I use a meta-analytical approach to combine the results of published studies reporting on the relationships between attractiveness-associated sire traits and various fitness-associated offspring traits. As a way of controlling for differential allocation, I test for differences in the magnitude of good genes effects between studies which used *in vitro* fertilization techniques (therefore preventing the possibility of differential maternal investment influencing offspring traits) and studies in which this process could potentially operate.

Throughout the rest of this chapter, the term 'paper' refers, traditionally, to a published journal article, whereas the term 'study' actually refers to a single estimate of an effect size (one paper may thus contain several 'studies' if several different effect sizes were estimated from it, *e.g.* due to measurement of more than one offspring trait). This terminology may be a little counter-intuitive but helps avoiding linguistic monstrosities.

Methods

Data collection and handling

I searched Web of Science using various combinations of following keywords: sexual selection, ornament, mate choice, female choice, female preferences, good genes, genetic benefits, indirect benefits, Fisherian benefits, offspring, handicap, run-away selection, heritability. I also conducted a cited reference search for papers citing Moller and Alatalo (1999).

To be included in the meta-analysis, studies had to satisfy the following criteria: (i) having analyzed the relationship between sire trait known or supposed to be target of female choice (traits used only for intra-sexual competition for mates were not taken into account) and offspring fitness-related trait (indices combining offspring with parental traits – e.g. measures of hatching success not controlling for the presence of unfertilized eggs or measures of offspring number at certain developmental stage not controlling for the number of offspring initially produced – were not taken into account); (ii) had been performed on species with non-resource based mating systems or had applied experimental setup that excluded the possibility of direct benefits influencing the observed relationships; (iii) statistical methodology had been clearly described and not seriously flawed (to my unpleasant surprise, a number of studies had to be excluded for violating the requirement of data independence; even more sadly, some of them came from recent papers published in high-impact journals), (iv) paternity had been experimentally controlled or genetically confirmed – or the frequency of extra-pair

offspring was known to be <15% in the population studied (<20% if the sample size was at least 200); (v) it was possible to determine the direction of the effect.

Studies were divided into two categories based on whether they applied a breeding scheme excluding the possibility of differential maternal allocation influencing the observed relationships (IVF studies, *i.e.*, using *in vitro* fertilization techniques) or not (non-IVF studies). Three types of offspring traits were considered: (1) sexually selected traits (sexual ornaments, attractiveness to mates or mating success), (2) viability (survival rates at any life-cycle stage) and (3) performance traits (life history, physiological or behavioral characters related to fitness).

Pearson's product-moment correlation coefficients were used as measures of effect size. The coefficients were either obtained directly from the papers or calculated from other statistics reported, using the equations from Coltman & Slate (2003) or Nakagawa & Cuthill (2007). To be used in further analyses, the coefficients were transformed to Fisher's Z values (a normalizing transformation):

$$Z = \frac{1}{2}\ln\left[\frac{1+r}{1-r}\right]$$

where r is a Pearson's correlation coefficient.

Sampling variance of each transformed effect size was calculated according to Borenstein *et al.* (2009) as:

$$mev = \frac{1}{n-3}$$

where n is the number of sires used to calculate the effect size. This measure of sampling variance has a simple intuitive interpretation – the level of uncertainty associated with an effect size estimate is assumed to be reversely proportional to the size of a sample it was calculated from. In the following meta-analyses, effect sizes are weighted by the inverse of their sampling variances (*i.e.* by n-3).

Statistical analyses

I tested for publication bias by inspecting funnel plots, analyzing a correlation between effect sizes and their weights, and via the trim and fill technique (Duval and Tweedie 2000). Trim and fill method estimates the extent of a publication bias against results unsupportive of the tested hypothesis and the influence this bias has on the overall mean effect size calculated by the meta-analysis. Studies were treated as independent data points for the publication bias analyses.

Meta-analysis was performed using generalized linear mixed models, fitted with the Markov Chain Monte Carlo method implemented in R package MCMCglmm (Hadfield 2010, R Development Core Team 2010). Vector of Fisher-transformed effect sizes (Z values) constituted a dependent variable and each Z value was weighted by the inverse of its sampling variance. Two random factors were specified: species ID, to account for variation in the effect sizes resulting from differences between species, and paper ID, to account for variation result-

ing from differences between published articles. Flat non-informative priors with a uniform low degree of belief across all parameters were set in all analyses. All models were fitted with the following parameters: number of iterations: 1 000 000, burn-in period: 50 000, thinning interval: 500; *autocorr* function was then used to check for autocorrelations.

I calculated an overall weighted mean effect size by running a model containing only an intercept, random factors, and a vector of sampling variances. I re-ran this analysis using a data set adjusted with the trim and fill technique (Duval and Tweedie 2000) to obtain an estimate of the overall weighted mean corrected for publication bias.

Then I included breeding procedure (two levels: IVF and non-IVF) and offspring trait type (three levels: sexually selected, performance and viability traits) as fixed factors in the model, to assess their influence on the effect size estimates. I assumed that the magnitude of publication bias should not depend on breeding procedure applied or offspring trait type considered and hence should not influence the estimated effects of these factors. Therefore, I performed this analysis on the unadjusted data set. The initial model included both fixed factors (without interaction). If a factor was evidently non-significant (P>0.2), it was subsequently removed from the model. An output table for fixed factors in MCMCglmm consists of an intercept, which is a posterior mean for the combination of (numerically or alphabetically) first levels of all fixed factors, tested against 0, and of the estimates of all other levels of the factors, tested against the intercept. For example, with factor levels coded as above, the estimated effect of 'breeding procedure' factor will refer to the difference between IVF and non-IVF studies in the mean effect size on sexually selected traits (offspring trait type 1); and the estimated effect of offspring trait type 'viability' will refer to the difference in the mean effect size between viability and sexually selected traits in IVF studies. Therefore, I run 6 different models representing every possible combination of factor levels in the intercept, before deciding to remove a factor.

Results

50 published papers were included in the meta-analysis, reporting on sire trait – offspring trait relationships in 35 different species. Number of studies (effect size estimates) per paper ranged from 1-8 and number of studies per species ranged from 1-14. In total, 119 effect sizes were collected (see Appendix). Only 8 out of 22 studies included in Moller and Alatalo's (1999) data set are also included here, since the remaining 14 studies did not satisfy all the criteria listed in Methods: *Data collection and handling*.

Initial analysis indicated that the overall mean effect size was not significantly different from 0 (r = 0.171; pMCMC = 0.321). However, a histogram of the residuals revealed the presence of an extreme outlier (study No 106 in the Appendix), which was removed from further analyses.

After removing the outlier, I obtained a highly significant positive estimate of an overall mean effect size (r = 0.268; pMCMC <0.001). However, a funnelplot of Z-transformed effect sizes against their weights indicated the presence of publication bias against studies with small sam-

ple sizes and negative effect sizes (Fig. 1). This was supported by a significant negative Spearman correlation between study weight and effect size (r_S = -0.253, P=0.006). The trim and fill analysis suggested that there were 4 effect sizes missing due to publication bias. After adjusting the data set by adding the inferred missing effect sizes (Duval and Tweedie 2000), Spearman correlation between study weight and effect size was still negative, but no longer significantly so (r_S = -0.144, P=0.114), indicating that the trim and fill procedure was a fairly effective way of correcting the publication bias in the data. The overall mean effect size estimated for the adjusted data set dropped slightly (from r= 0.268 to r=0.235) but remained highly significant (pMCMC < 0.001).

In the model including fixed factors, breeding procedure did not significantly affect effect size (P > 0.7 in each of the 6 models, see Table 1 for an example) and was therefore removed from further analysis. The resulting model indicated that the differences between offspring trait categories were not significant either, although the difference between sexually selected and viability traits approached significance: viability traits tended to be associated with smaller effect sizes than sexually selected traits (Tables 2 and 3).

Differences between species had significant influence on the variance among the effect sizes, as manifested by increased values of Deviance Information Criterion of the models with 'species ID' random factor excluded (model without fixed factors: DIC = -126.7182 when species was included in the model, DIC= -105.0806 when species was not included; model with 'offspring' fixed factor: DIC = -144.273 when species was included, DIC = -137.22 when species was not included).

Figures and Tables

Figure 1. Z-transformed effect sizes plotted against their weights (n-3, where n is a number of sires used in a study). Weighted mean effect size is marked with the vertical line.

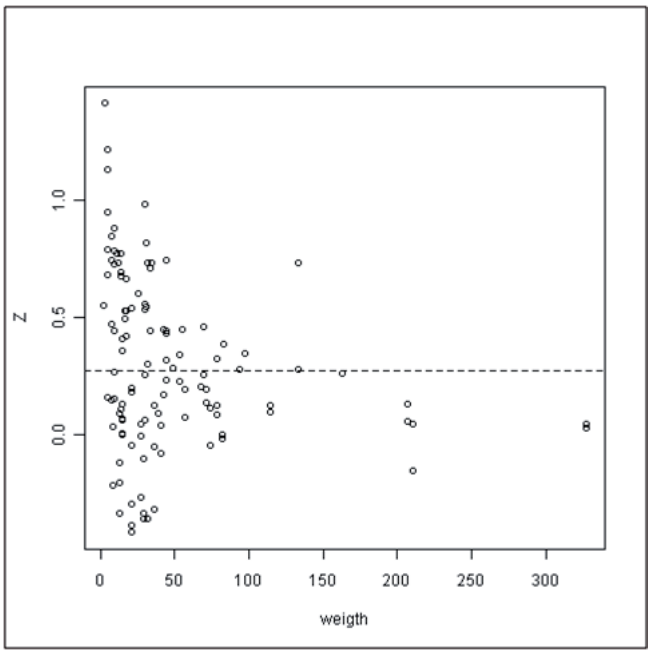

Table 1. Results of a generalized linear mixed model meta-analysis (random factors: paper ID and species ID), estimating the influence of breeding scheme and offspring trait type on the magnitude of study effect sizes.

Effect	Posterior mean	Confidence interval			pMCMC
Intercept	0.403	0.134	-	0.660	0.001
Breeding scheme (non-IVF)	-0.044	-0.303	-	0.178	0.743
Offspring trait type (Viability)	-0.180	-0.404	-	0.025	0.097
Offspring trait type (Performance)	-0.133	-0.318	-	0.057	0.169
Note: N = 118 studies					

Table 2. Results of a generalized linear mixed model meta-analysis (random factors: paper ID and species ID), estimating the influence of offspring trait type ('breeding scheme' factor was excluded from this model) on the magnitude of study effect sizes.

Effect	Posterior mean	Confidence interval			pMCMC
Intercept	0.358	0.217	-	0.505	0.000
Offspring trait type (Viability)	-0.164	-0.358	-	0.028	0.105
Offspring trait type (Performance)	-0.114	-0.288	-	0.056	0.188
Note: N = 118 studies					

Table 3. Weighted mean effect sizes for three categories of offspring traits, estimated using a generalized linear mixed model meta-analysis (random factors: paper ID and species ID).

Offspring trait type	Posterior mean	Confidence interval			pMCMC
Sexually selected	0.345	0.229	-	0.518	0.000
Performance	0.236	0.112	-	0.390	0.000
Viabillity	0.193	0.029	-	0.349	0.019
Note: $N_{Sexually\ selected} = 47$; $N_{Performance} = 49$; $N_{Viability} = 23$					

Discussion

I found a moderate, but highly significant, positive average correlation between attractiveness-related sire traits and fitness-related progeny traits. This result is consistent with previous meta-analysis by Moller and Alatalo (1999), who found a positive association of male

sexual traits with offspring viability. In this chapter, after excluding an outlier showing unusually large negative correlation (study No 106 in the Appendix), the mean effect size for viability was slightly higher (r = 0.193, without correcting for publication bias) but not significantly different from that obtained by Moller and Alatalo (r = 0.122 which is within the confidence interval of my estimate – see Table 3). Here, I additionally considered other progeny traits, since association with only one fitness component might not reflect association with total fitness. The effect sizes tended to be stronger for sexually selected than for viability and performance-related offspring traits, but these differences were not statistically significant.

Furthermore, effect sizes reported by Moller and Alatalo (1999) could potentially be confounded with increased allocation of resources by females mated to more attractive males (Cunningham and Russel 2000, Sheldon 2000). However, I did not find any evidence for differential maternal allocation affecting the magnitude of estimated good genes effects: mean effect sizes did not differ between studies that controlled for differential allocation from those that did not. Unfortunately, this particular result is difficult to interpret due to potentially confounding taxonomic effects: with the exception of one paper looking at one species (*Gallus gallus*), all the studies using IVF for breeding were done on amphibians or ray-finned fishes, whereas the non-IVF group consists of studies performed on much wider range of taxa (see Appendix). Since the majority of studies included in this meta-analysis did not apply breeding methods preventing maternal effects from confounding the observed results, the estimated correlations should be interpreted with caution – even though some authors tested and did not find any evidence for differential allocation (Hoefler et al. 2009, Petrie 1994), and some have actually found evidence for increased maternal investment in the progeny of unattractive males (Byers and Waits 2006). Further work is needed to estimate the contribution of differential allocation to the observed associations between sire attractiveness traits and offspring fitness components.

It would be tempting to say that the average correlation between sire traits and offspring fitness-related traits calculated above provides an estimate of the mean genetic correlation between male display and total fitness across different animal taxa. However, several lines of reasoning urge great caution with such claims.

First, the average was calculated across a wide range of fitness-related traits. I grouped them into three categories (sexually selected, viability and performance traits, see Methods: *Data collection and handling*) and each category still included a variety of different traits. Those traits are likely to differ enormously in the strength of their relationship with total fitness. The mean effect size calculated from this meta-analysis may therefore overestimate the genetic correlation of interest if the traits associated with larger effect sizes tend to be less strongly related to fitness – or conversely, underestimate it if the opposite is true. Therefore, effect size of each study should ideally be weighted by a measure of correlation between fitness and offspring trait analyzed in the study. Unfortunately, such data are impossible to obtain for most studies.

Second, I found evidence for publication bias. Even though the trim and fill analysis suggested that it was not a very severe problem in this data set, it remains an important issue to consider in every meta-analysis. A complementary way of assessing and correcting for publication bias is to include unpublished studies in a meta-analysis alongside the published ones, and to compare the mean effect sizes between those two sets of studies. I am therefore planning to e-mail requests for unpublished data to two widely subscribed mailing lists: EvolDir (http://evol.mcmaster.ca/evoldir.html) and ECOLOG (http://www.lsoft.com/scripts/wl.exe?SL1=ECOLOG-L&H=LISTSERV.UMD.EDU) and individually to scientists studying sexual selection.

Third, the potential confounding effects of differential maternal allocation are yet to be estimated, as discussed above.

Bearing in mind these cautionary remarks, the moderately strong and highly significant positive correlation between sire attractiveness traits and offspring fitness-related traits nevertheless indicates that the genetic correlation between male attractiveness and fitness is likely to be positive, which is a necessary prerequisite for the "good genes" selection on female preferences to operate (Kirkpatrick and Barton 1997). Relative importance of the good genes process for the origin and maintenance of female preferences will depend on strength and direction of other evolutionary forces acting upon them – particularly on the costs of mate choice. Even weak indirect selection will be important if preference alleles are selectively neutral (Kirkpatrick and Barton 1997), whereas stronger positive selection will be needed if they inflict considerable direct costs on females, as seems to be the case in some systems (e.g. Pitnick and Garcia-Gonzalez 2002). On the other hand, positive direct effects of choice, particularly in terms of fertility assurance (e.g. Cotton et al. 2010) or avoiding excessive mating (Gavrilets et al. 2001) may be more common, and therefore more important for preference evolution, than previously realized.

Chapter 4

Age, virginity and sex – do female bulb mites prefer young males as mating partners?

Introduction

The traditional view derived from models of age-specific female preferences has been that old age is an indication of high genetic quality of males that have demonstrated their ability to survive, whereas cohorts of young males include individuals of both high and low viability (Manning 1985; Brooks and Kemp 2001). Thus, mating with older males should benefit females by increasing viability of their offspring. However, what counts from the perspective of a female "shopping for good genes" for her progeny is not the quality of her mate, but of his gametes (Beck and Promislow 2007). There are several reasons to believe that the latter may actually decline with male age in many species. For one, the number of deleterious germline mutations is likely to increase with age if stem cells continue to divide after an organism has reached sexual maturity, which is the case in many species (Drost and Lee 1995; Hansen and Price 1999). This process will lead to a decreased fitness of older males' offspring. Other processes, such as weaker selection on older individuals or trade-offs between late-age and early-age life history parameters can also result in a decline in male breeding value over lifetime (Hansen and Price 1995). Moreover, male fertility can also decrease with age, inferring a direct cost to females mating with older males.

Fitness consequences of mating with males of particular age classes will thus depend on specific life history parameters (Kokko 1998; Beck and Powell 2000) and on the number of divisions a male germline undergoes after he reaches sexual maturity (Radwan 2003). In humans, paternal age has been shown to affect the incidence of a number of genetic disorders (Crow 2000; Sartorius and Nieschlag 2010). Negative effects of male age on offspring fitness and/or female fertility have also been shown in other species. Prokop et al. (2007) found that in bulb mite, daughters of 4- to 5-week-old sires had a 6% lower fecundity than those of 4- to 8-day-old sires. Serre and Robaire (1998) reported a significantly higher neonatal death rate in the progeny of older Norway rat males. In *Drosophila melanogaster*, Price and Hansen (1998) reported a 3% decrease in larval viability and a 4–6% decrease in male mating ability in the progeny of 34-day-old fathers compared to 2- and 14-day-old ones, whereas Long and Pischedda (2005) reported a significantly lower reproductive success under competition in sons of 13-day-old males compared to those of 1-day-old males. In barn swallows, chick body size and feather development were negatively influenced by sire age, which accounted for 2–3.5% of the variance in those traits (Saino et al. 2002). Jones et al. (2000) observed a higher egg hatching success in lekking sandfly females mated to young and middle aged than to old males, which could reflect either lower quality of zygos sired by old fathers or the old males' lower fertilization

rate (Jones et al. 2000). Decreased fertility of old males was observed in domestic fowl, with 7-year-old males having reduced probability of semen transfer, fewer sperm in ejaculate and lower sperm velocity than 2- to 3-year-old ones (Dean et al. 2010).

On the other hand, (Priest et al. 2002) found a significant positive relationship between sire age and offspring longevity in *D. melanogaster,* whereas Pervez et al. (2004) reported that in a predatory ladybird beetle, eggs sired by 20- to 30-day-old males had significantly higher viability than those sired by younger males. The latter result could have been due to decreased embryo mortality or increased fertility rate of older males – an effect that has also been observed in a stalk-eyed fly *Cyrtodiopsis whitei* (Wilkinson and Sanchez 2001). Hegyi et al. (2006) found that subadult males produced slower growing progeny than adult ones (controlling for direct effects by cross-fostering); however, they did not analyze the influence of adult fathers' age on their offspring growth. In some species, male reproductive performance and/or progeny fitness may in fact be a non-linear function of age. In common lizard, offspring sired by younger and older males had lower survival than those sired by medium-aged ones (Richard et al. 2005). Highest fertility rate of females mated to intermediate-age males was found in hide beetle (Jones and Elgar 2004; Hale et al. 2008). In the European corn borer, females paired to 3-day-old males were more fecund, lived longer, and had a longer oviposition period than those paired to older (6- and 9-days-old) or newly emerged males (Milonas and Andow 2010); decline in longevity was particularly strong in mates of old males.

In species where older males sire lower quality offspring or otherwise negatively affect fitness of their mates, evolution of female preferences towards younger mates may be expected (Beck and Promislow 2007). As yet, the evidence for such preferences is scarce. (Verburgt et al. 2011) reported that female field crickets choose the song of young males over that of old males. (Prokop et al. 2007) found that old bulb mite males were less likely to mate within an hour of observation than were the young males; however, female preference *versus* male mating ability effects could not be unequivocally distinguished in that experiment. Female bushcrickets were found to discriminate against older males (Wedell and Ritchie 2004); however, the authors attributed this effect to age-based differences in the amount of resources provided to a female at mating, rather than to differences in male genetic quality. Female discrimination against old males was also observed in the lekking sandfly (Jones et al. 2000) and the hide beetle (Jones and Elgar 2004); however, in both systems, females in fact preferred middle-aged males to both old and young ones. Milonas and Andow (2010) did not find any indication of female discrimination against old males in European corn borer, although mating with such males entailed significant fitness costs. In a range of species, positive relationships between male age and female preferences have actually been reported, for example in great bustards (Alonso et al. 2010), thornbug treehoppers (De Luca and Cocroft 2008), western bluebirds (Dickinson 2001), guppies (Miller and Brooks 2005) or Bullock's orioles (Richardson and Burke 1999). Additionally, in some species increased mating success of older males was

observed that was not unequivocally ascribed to male or female effects. For example, older males obtained more copulations in the Mexican fruit fly (Perez-Staples et al. 2010), were more likely to pair in red-breasted flycatcher (Mitrus 2006), and attained more extra-pair fertilizations in red-wing blackbirds (Weatherhead and Boag 1995), blue tits (Delhey et al. 2006), and reed buntings (Suter et al. 2009).

Rhizoglyphus robini (Acari: Acaridae) is well suited to test the effects of male age. It is relatively long living for such a small species, with adult males surviving for up to 2 months (Radwan and Bogacz 2000). The species is highly promiscuous and reproduces continually, which implies that spermatogenesis must be intense throughout adulthood and older males must have undergone a large number of germline divisions (W. Witaliński, personal communication). Hence, the mutation load in sperm is likely to increase with male age. Old males sire daughters with decreased fecundity, and are less likely to mate within 1 – 1.5 hour of observation (Radwan et al. 2005; Prokop et al. 2007). Furthermore, old males lose in sperm competition with young ones (Radwan et al. 2005), which suggests that polyandry may be an efficient way of decreasing the proportion of offspring sired by older partners (Radwan 2003). However, multiple copulations decrease female fecundity in this species (Kołodziejczyk and Radwan 2003) and hence unnecessary re-matings should be avoided by females. If females can assess male age before copulation, the decision to re-mate should be contingent on the age of previous versus potential new partner. Prokop et al. (2007) found that females previously mated to young males are less likely to engage in copulations than virgins, regardless of the new partner's age. However, the behavior of females previously mated to old males was not examined in that study.

In this study, two categories of non-virgin females were used: ones that had previously copulated with young males and ones that had previously copulated with old males. Virgin females were used as a third category for additional comparisons. I predicted that females previously paired with an old partner will be more likely to copulate with a young than with an old male (manifested by a significant effect of an interaction between the age of a female's previous and new partner on mating probability), and more so than females previously mated to a young partner (manifested by a significant effect of the age of a female's previous partner on mating probability). I also predicted that virgin females will be more likely to copulate than both types of mated females.

The second aim of the study was to collect detailed behavioral data that would allow us to determine whether the lower probability of copulating by older males (Radwan et al. 2005, Prokop et al. 2007) indeed results from female resistance to such mates or whether it is simply an effect of male aging and a consequent decrease in mate securing ability. If the latter was true, I expected that male aging should result in a decreased number and/or efficiency of mating attempts.

Methods

Study animals and rearing conditions

Mites used in this study were from a stock culture derived from a colony of about 200 individuals found on onions in a garden near Kraków in 1998, and kept in the laboratory as a large population (>1000 individuals, subdivided into 6 subpopulations mixed once a month) for about 550 generations before the commencement of this project. The subpopulations were kept in jars 2.5 cm in diameter and 2 cm high, maintained at 23-26 °C, >90% humidity, and fed once a week with powdered yeast and wheat germ *ad libitum*. Once a month about a quarter of the food and debris, containing several hundred mites at different stages of development, were transferred to fresh jars. The same feeding, humidity, and temperature conditions were maintained throughout all the experiments described below. Individually isolated mites, pairs, and small groups of mites were kept in glass tubes 0.8 cm in diameter and 2 cm high with plaster of Paris bases soaked with water and were provided with food *ad libitum*.

Male cohorts

The experiment involved 2 male cohorts. Young males were taken for experiments 4-7 days after emergence and old males 30–40 days after emergence. The old male cohort was established *ca*. 4 weeks in advance of the young male cohort. Each cohort was obtained by placing about 300 main stock females in 3 Petri dishes 9 cm in diameter, lined with moist tissue paper (about 100 females per dish). Females were removed after 24 hours and the dishes were left for 5 days to let the eggs hatch. After that, food was provided regularly to developing larvae. On day 12, tritonymphs (the last nymphal instar) were isolated individually to separate tubes. On days 15-17, newly emerged adults were sexed and 2 females were added to each tube containing a male. In the old male treatment, males were kept with females for 30–40 days and transferred into new tubes every 7-10 days (before the offspring produced developed into adults) to avoid confusing them with newly emerged males. Females were replaced if necessary. In the young male treatment, males were kept with females for 3–8 days. As matings occur at the frequency of about 8 per day (Radwan and SivaJothy 1996) and previous partners are not discriminated against (Konior et al. 2001), young males had mated on average >30 times before they were used for experiments. Nevertheless, apart from age, male cohorts differed in the number of matings when used in the experiments. However, apart from giving young males opportunity to deplete any sperm reserves they might have had at virginity, I chose not to control for male mating history because in nature male age and the number of his matings are tightly correlated. Thus, any effects of male age in his performance and attractiveness can result from his mating history as well as from aging *per se*.

Two male morphs occur in *Rhizoglyphus robini* (heteromorphic fighters, possessing a thickened and sharply terminated third pair of legs, and homeomorphic scramblers with unmodified legs), and both male cohorts included fighter and scrambler morphs.

Female mating status

The experiment involved 3 female groups: virgins, females previously mated to a young male (young male–mated) and females previously mated to an old male (old male–mated). All females were obtained by isolating tritonymphs and were 4-7 days old when used in the experiments. In the virgin female treatment, females had been kept individually in the tubes until the commencement of the experiment. In the young male–mated female treatment, each female had been kept with a male of the same age for 4-7 days. In the old male–mated female treatment, each female had been kept with a 30-40 days old male for 4-7 days.

Experimental treatment

The behavior of 149 pairs was observed: 19 virgins paired to old males and 19 paired to young males; 26 old male-mated females paired to old males and 34 paired to young males; 27 young male-mated females paired to old males and 24 paired to young males. Observations were made under a stereomicroscope, in glass tubes with plaster of Paris bases and no food provided. Each pair was observed for 90 minutes. A female was placed in a tube first. After *ca.* 5 minutes, a male was added and his motility was measured for 3 minutes unless there had been a physical contact between the mites before this time passed; in such cases the motility measurement was stopped. Motility was assessed as a proportion of the measurement time that a male spent moving. Latency to the first mounting attempt by a male, number of such attempts and the occurrence of copulation (yes/no) were subsequently recorded.

Statistical analyses

Statistical analyses were performed in R 2.12.1 (R Development Core Team, 2010). I analyzed the influence of female mating status and male age on the probability that a male attempted to mount a female, the latency to first such attempt, the probability of copulation and the number of male mounting attempts (this last variable was only analyzed for pairs that did not eventually copulate). Female mating status effect was tested using two *a priori* contrasts: (1) female virginity, distinguishing virgin from mated females and (2) mated female class, distinguishing old male-mated from young male-mated females. Male morph and pre-contact motility (a continuous variable) were added as additional explanatory variables in each model.

I subsequently ran another two models for the probability of copulation: one with the latency to first mount and one with the number of male mating attempt as a covariate, excluding all pairs where a male did not attempt to mount a female at all. The number of mating attempts had a strongly skewed distribution (Fig. 1). Therefore I transformed it into an ordinal scale variable which I called ‘male mounts rank’: males that performed only one attempt were given rank =1, those performing 2-3 attempts got rank =2 and those performing > 3 (4-16) attempts got rank =3.

Each model initially included all possible interactions, which were subsequently removed from a model when non-significant (threshold P value for removal = 0,1). The only exception was made for the female status × male age interactions – these were always retained in the final model because they were testing one of the main hypotheses.

Results

Probability of mounting was rather high, with almost 80% of males attempting to mount a female at least once during 1.5 h observation, and it was not significantly affected by any independent variable (Table 1). Latency to first mounting attempt was significantly influenced by male age and female virginity, with young males mounting and virgin females being mounted earlier than old males and mated females, respectively (Table 2). Male pre-contact motility and the number of unsuccessful mounting attempts (the latter was only analyzed for a subset of pairs that did not copulate within the observation time) were not significantly affected by any independent variable, although the effect of male morph approached significance in both models, with scrambler morphs being more active (Table 3) but performing fewer mounting attempts (Table 4).

Only about 32% of pairs mated within the observation time. Probability of copulation was significantly affected by male age and motility – young males were more likely to copulate (Table 5, Fig. 2) whereas male motility decreased the probability of successful mating (Table 5). In order to compare the current results with those obtained in an earlier study (Prokop et al. 2007), I re-ran the analysis of copulation probability on the subset of data excluding old male-mated females. The results did not differ qualitatively from those obtained for the full dataset, although the effect of female mating status approached significance, with virgin females being more likely to mate (Table 6, Fig. 2).

After including male mounts rank (3-rank index of the number of mountings, see Methods: Statistical analyses) in the model for copulation probability, the effect of male age remained significant, whereas the effect of male motility did not. The effect of male mounts rank neared significance and surprisingly, it was negative, with more 'persistent' males being less successful at mating (Table 7). Latency to first mounting did not significantly affect mating probability (Table 8).

Figures and Tables

Figure 1. Frequency distribution of the number of mounting attempts by males (data for males that performed at least 1 mount are shown).

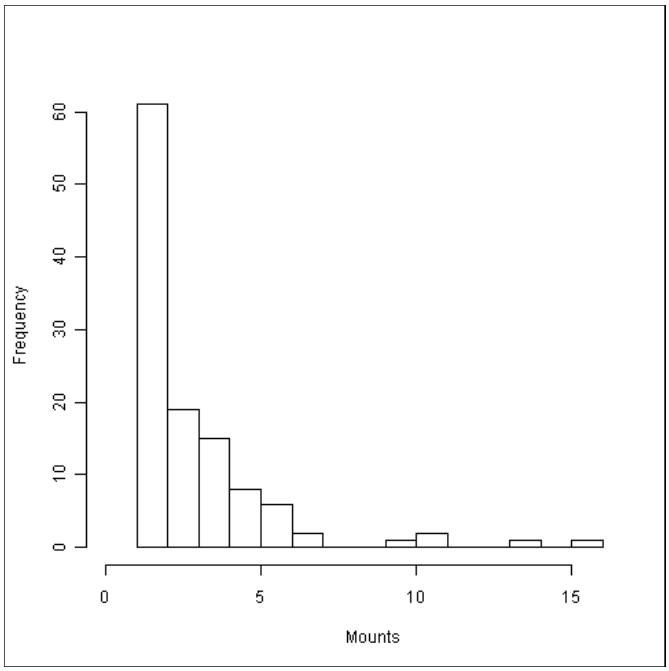

Figure 2. Copulation probability (y axis) in relation to male age (old *vs* young), female mating status (virgin *vs* old male-mated *vs* young male-mated) and male morph (fighter *vs* scrambler).

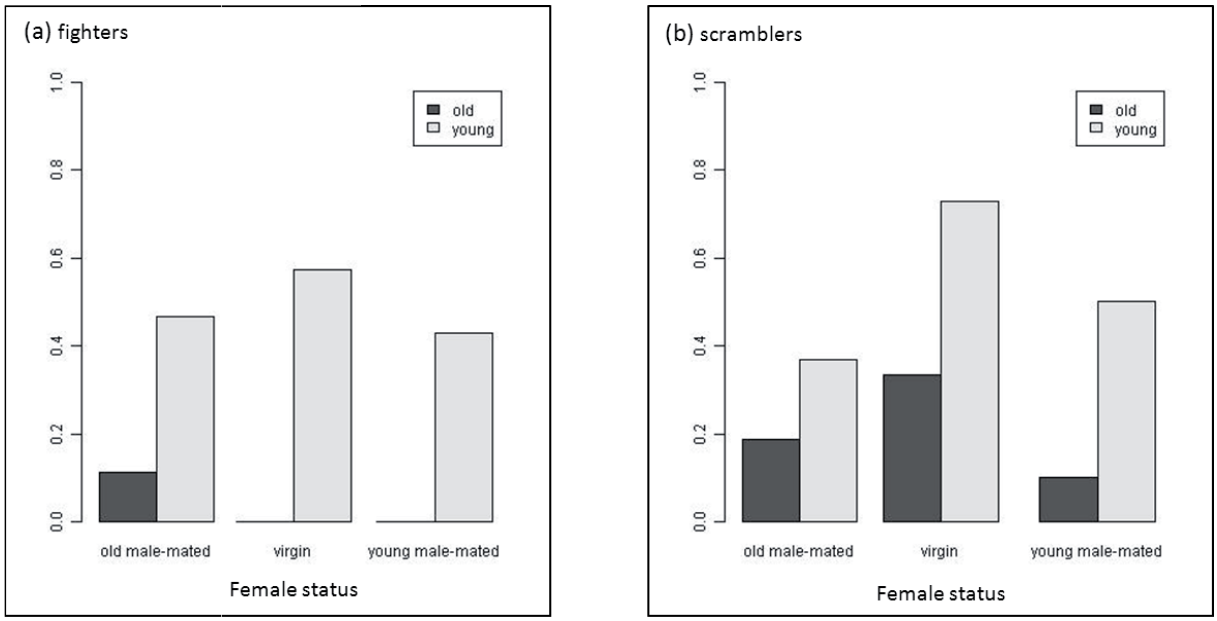

Table 1. Results of a generalized linear model (binomial error distribution, logit link function) for the probability of at least one mounting attempt by a male.

Effect	Estimate	SE	Z value	P
Intercept	2.30	1.26	1.82	0.068
Male age: young	-0.07	0.45	-0.15	0.880
Female virginity (Virgin)	0.10	0.24	0.40	0.687
Mated female class (Young male-mated)	0.17	0.34	0.48	0.631
Male morph (Scrambler)	-0.75	0.48	-1.56	0.118
Male motility	-0.34	1.39	-0.25	0.806
Male age (Young) × Female virginity (Virgin)	0.22	0.36	0.60	0.552
Male age (Young) × Mated female class (Young male-mated)	-0.37	0.46	-0.79	0.430
Residual deviance:	143.75 on 140 degrees of freedom			

Table 2. Results of a general linear model for the latency to first mounting attempt by a male.

Effect	Estimate	SE	t value	P
Intercept	3234.55	616.74	5.25	0.000
Male age: young	-841.22	258.48	-3.25	0.002
Female virginity (Virgin)	-337.61	133.53	-2.53	0.013
Mated female class (Young male-mated)	224.98	211.27	1.07	0.289
Male morph (Scrambler)	-207.02	266.51	-0.78	0.439
Male motility	-603.75	707.12	-0.85	0.395
Male age (Young) × Female virginity (Virgin)	73.82	188.89	0.39	0.697
Male age (Young) × Mated female class (Young male-mated)	-212.22	298.01	-0.71	0.478
Residual standard error:	1362 on 110 degrees of freedom			

Table 3. Results of a general linear model for male pre-contact motility.

Effect	Estimate	SE	t value	P
Intercept	0.82	0.03	28.83	0.000
Male age: young	0.02	0.03	0.79	0.431
Female virginity (Virgin)	0.00	0.02	-0.12	0.902
Mated female class (Young male-mated)	-0.01	0.02	-0.61	0.546
Male morph (Scrambler)	0.06	0.03	1.92	0.057
Male age (Young) × Female virginity (Virgin)	0.01	0.02	0.24	0.815
Male age (Young) × Mated female class (Young male-mated)	0.00	0.03	0.11	0.916
Residual standard error:	0.1708 on 141 degrees of freedom			

Table 4. Results of a generalized linear model (quasipoisson error distribution, log link function) for the number of male mounting attempts.

Effect	Estimate	SE	t value	P
Intercept	0.88	0.67	1.31	0.193
Male age: young	0.33	0.20	1.64	0.104
Female virginity (Virgin)	0.13	0.10	1.32	0.190
Mated female class (Young male-mated)	-0.07	0.16	-0.42	0.673
Male morph (Scrambler)	-0.35	0.20	-1.74	0.085
Male motility	0.08	0.74	0.11	0.910
Male age (Young) × Female virginity (Virgin)	0.22	0.14	1.53	0.129
Male age (Young) × Mated female class (Young male-mated)	-0.10	0.25	-0.39	0.700
Residual deviance:	213.81	on 90	degrees of freedom	

Table 5. Results of a generalized linear model (quasibinomial error distribution, logit link function) for copulation probability.

Effect	Estimate	SE	t value	P
Intercept	0.01	1.11	0.01	0.990
Male age: young	1.99	0.46	4.33	0.000
Female virginity (Virgin)	0.34	0.25	1.34	0.183
Mated female class (Young male-mated)	-0.53	0.48	-1.11	0.268
Male morph (Scrambler)	0.52	0.43	1.20	0.234
Male motility	-2.59	1.26	-2.05	0.042
Male age (Young) × Female virginity (Virgin)	-0.04	0.32	-0.13	0.894
Male age (Young) × Mated female class (Young male-mated)	0.62	0.55	1.11	0.267
Residual deviance:	150.12	on 136	degrees of freedom	

Table 6. Results of a generalized linear model (binomial error distribution, logit link function) for copulation probability, fitted to data without the old male-mated female category.

Effect	Estimate	SE	Z value	P
Intercept	1.11	1.75	0.63	0.528
Male age: Young	2.02	0.81	2.51	0.012
Female status: Young male-mated	-1.72	1.03	-1.68	0.094
Male morph: Scrambler	1.00	0.62	1.61	0.108
Male motility	-3.48	1.83	-1.90	0.058
Male age (Young) × Female status (Young male-mated)	0.82	1.22	0.67	0.504
Residual deviance:	81.261	on 79	degrees of freedom	

Table 7. Results of a generalized linear model (quasibinomial error distribution, logit link function) for copulation probability, fitted with male mounts rank added as an additional covariate.

Effect	Estimate	SE	t value	P
Intercept	0.23	1.33	0.17	0.864
Male age: young	2.25	0.53	4.26	0.000
Female virginity (Virgin)	0.31	0.29	1.08	0.281
Mated female class (Young male-mated)	-0.52	0.52	-1.00	0.319
Male morph (Scrambler)	0.37	0.51	0.73	0.465
Male motility	-1.56	1.43	-1.09	0.279
Male mounts rank	-0.53	0.30	-1.76	0.082
Male age (Young) × Female virginity (Virgin)	-0.11	0.37	-0.29	0.775
Male age (Young) × Mated female class (Young male-mated)	0.63	0.62	1.02	0.309
Residual deviance:	116.57 on 104 degrees of freedom			

Table 8. Results of a generalized linear model (quasibinomial error distribution, logit link function) for copulation probability, fitted with mating latency added as an additional covariate.

Effect	Estimate	SE	t value	P
Intercept	0.19	1.37	0.14	0.890
Male age: young	2.05	0.53	3.86	0.000
Female virginity (Virgin)	0.19	0.29	0.67	0.502
Mated female class (Young male-mated)	-0.38	0.51	-0.74	0.460
Male morph (Scrambler)	0.48	0.49	0.98	0.329
Male motility	-2.15	1.38	-1.56	0.121
Mating latency	0.00	0.00	-0.92	0.358
Male age (Young) × Female virginity (Virgin)	-0.05	0.36	-0.13	0.899
Male age (Young) × Mated female class (Young male-mated)	0.49	0.61	0.80	0.423
Residual deviance:	119.62 on 105 degrees of freedom			

Discussion

The 'trade-up' hypothesis predicts that in polyandrous species a female should base her re-mating decision on the quality of her previous as well as a (potential) new partner: when the initial constraints prevent her from mating with the most preferred male, she should 'trade up' and re-mate with a superior male if given a chance (Jennions and Petrie 2000). Such be-

havior has been observed in a cricket *Gryllus bimaculatus*: in a sequential pairing experiment, mated females were generally more likely to reject males than virgins, but the opposite was true when the second male was larger than the first (Bateman et al. 2001); but not in bank voles, where females were shown to re-mate frequently and indiscriminately with respect to male dominance status (Klemme et al. 2006). In some species – e.g. smooth newts (Gabor and Halliday 1997), guppies (Pitcher et al. 2003), or harlequin beetle-riding pseudoscorpions (Zeh and Zeh 2007) – previously mated females have been shown to be more choosy than virgins, suggesting that females are indiscriminative at their first mating to ensure fertilization of their eggs but subsequently mate preferentially with males of higher quality (Pitcher et al. 2003).

Based on a previous finding of decreased fecundity in old males' daughters (Prokop et al. 2007), I had predicted that bulb mite females previously copulating with old males should be more likely to re-mate, especially when paired with a young male. Given the last male sperm precedence (Radwan 1997) and higher competitiveness of sperm of younger males (Radwan et al. 2005), old male-mated females re-mating with a young partner would ensure that a high proportion of their eggs is fertilized by the latter. However, I found no evidence for such 'trade up' female behavior: the probability of copulation was not significantly influenced by the age of the previous partner of the female, or by its interaction with the age of her new partner (Table 5, Fig. 2). Also, I did not find any effect of an interaction between male age and female virginity (a contrast distinguishing virgin from mated females) on copulation probability (Table 5, Fig. 2), which is consistent with previous findings (Prokop et al. 2007). Therefore, my results do not provide evidence for bulb mite females 'trading up' with respect to male age.

Contrary to earlier results (Prokop et al. 2007) I did not observe a significantly higher copulation probability of virgin than mated females (Table 5). However, when I excluded old male-mated females from the analysis in order to obtain results directly comparable with those from the previous study (in which I only used virgin and young male-mated female categories), the effect of female status neared significance, with virgin females tending to be more likely to mate (Table 6, Fig. 2).

I also found a significant effect of female mating status on the latency to first mounting attempt by a male – specifically, virgins were approached sooner than non-virgins. This may indicate that virgin females advertise their presence more than mated females in order to ensure fertilization of their eggs, and this causes males to locate them sooner. Indeed, bulb mite females use pheromones to attract males (Mizoguchi et al. 2003), although it remains to be tested whether virgin females use them more frequently or in higher concentration. However, mounting latency did not predict mating success in this experiment (tab. 6, see also below) so the interpretation of this effect is difficult.

In accordance with previous findings (Radwan et al. 2005, Prokop et al. 2007), male age decreased the probability of mating. One of the aims of the current study was to find out whether this was due to female discrimination against older partners or to a decline in mate

securing ability with male age. I found that old males indeed took longer to approach a female (longer latency to first mounting attempt, Table 2) and tended to perform fewer mounting attempts, although the latter effect was not statistically significant (Table 4). However, these differences in behavior between male cohorts did not explain the difference in copulation probability. Mounting latency was unrelated to the probability of mating (Table 8) whereas male mounts rank had in fact a negative effect, although it did not reach statistical significance (Table 7). This would suggest that females have more control over mating than males do and that decreased probability of copulation by older males results from female resistance rather than age-based differences in male mating propensity.

To summarize the main findings of this experiment, I did not find evidence of female bulb mites 'trading up' with respect to male age, either by becoming more discriminative once they ensured fertility or by being more willing to re-mate with a young partner if they previously copulated with an old one. I did, however, confirm that mating probability was negatively related to male age and this effect could not be explained by age-based differences in male behavior, therefore suggesting that it was female-driven.

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Appendix. Sexual selection studies.

Information on reference, taxonomy, type of breeding scheme (IVF - excluding the possibility of differential allocation influencing the estimated correlation or non-IVF with confounding effects of differential allocation possible), offspring trait and assigned trait type, number of sires used in a study and estimated effect size (Pearson's r).

No	Reference	Species	Class	Breeding scheme	Offspring trait	Offspring trait type	Number of sires	r
1	Barber et al. 2001	*Gasterosteus aculeatus*	Actinopterygii	IVF	growth	performance	30	-0.259
2	Barber et al. 2001	*Gasterosteus aculeatus*	Actinopterygii	IVF	physiological	performance	18	0.345
3	Huuskonen et al. 2009	*Coregonus lavaretus*	Actinopterygii	IVF	behavioral	performance	10	0.633
4	Petersson and Jarvi 2007	*Salmo trutta*	Actinopterygii	IVF	behavioral	performance	18	0.006
5	Petersson and Jarvi 2007	*Salmo trutta*	Actinopterygii	IVF	behavioral	performance	18	0.069
6	Petersson and Jarvi 2007	*Salmo trutta*	Actinopterygii	IVF	behavioral	performance	18	0.389
7	Petersson and Jarvi 2007	*Salmo trutta*	Actinopterygii	IVF	growth	performance	18	0.001
8	Pitcher and Neff 2007	*Oncorhynchus tshawytscha*	Actinopterygii	IVF	growth	performance	11	0.033
9	Doty and Welch 2001	*Hyla versicolor*	Amphibia	IVF	growth	performance	10	0.150
10	Doty and Welch 2001	*Hyla versicolor*	Amphibia	IVF	behavioral	performance	10	0.442
11	Mitchell 1990	*Bufo woodhousei*	Amphibia	IVF	adult size	performance	20	0.583
12	Welch 2003	*Hyla versicolor*	Amphibia	IVF	adult size	performance	24	0.181
13	Welch 2003	*Hyla versicolor*	Amphibia	IVF	adult size	performance	24	0.493
14	Welch 2003	*Hyla versicolor*	Amphibia	IVF	development	performance	24	-0.392
15	Welch 2003	*Hyla versicolor*	Amphibia	IVF	development	performance	24	-0.368
16	Woodward et al. 1988	*Hyla crucifer*	Amphibia	IVF	adult size	performance	18	0.064
17	Woodward et al. 1988	*Hyla crucifer*	Amphibia	IVF	development	performance	18	0.006

No	Reference	Species	Class	Breeding scheme	Offspring trait	Offspring trait type	Number of sires	r
18	Woodward et al. 1988	*Hyla crucifer*	Amphibia	IVF	growth	performance	18	0.133
19	Parker 2003	*Gallus gallus*	Aves	IVF	condition	performance	74	0.139
20	Parker 2003	*Gallus gallus*	Aves	IVF	condition	performance	74	0.191
21	Parker 2003	*Gallus gallus*	Aves	IVF	condition	performance	72	0.255
22	Parker 2003	*Gallus gallus*	Aves	IVF	condition	performance	72	0.432
23	Barber et al. 2001	*Gasterosteus aculeatus*	Actinopterygii	IVF	survival	viability	30	-0.003
24	Barber et al. 2001	*Gasterosteus aculeatus*	Actinopterygii	IVF	survival	viability	30	0.045
25	Jacob et al. 2010	*Salmo trutta*	Actinopterygii	IVF	survival	viability	19	0.461
26	Jacob et al. 2010	*Salmo trutta*	Actinopterygii	IVF	survival	viability	19	0.484
27	Pitcher and Neff 2007	*Oncorhynchus tshawytscha*	Actinopterygii	IVF	survival	viability	11	-0.214
29	Wedekind et al. 2008	*Coregonus zugensis*	Actinopterygii	IVF	survival	viability	10	0.690
30	Wedekind et al. 2008	*Coregonus zugensis*	Actinopterygii	IVF	survival	viability	8	0.160
31	Sheldon et al. 2003	*Rana arvalis*	Amphibia	IVF	survival	viability	20	0.400
32	Brooks 2000	*Poecilia reticulata*	Actinopterygii	non-IVF	reproductive	performance	39	0.129
33	Brooks 2000	*Poecilia reticulata*	Actinopterygii	non-IVF	reproductive	performance	33	0.491
34	Reynolds and Gross 1992	*Poecilia reticulata*	Actinopterygii	non-IVF	growth	performance	17	0.590
35	Reynolds and Gross 1992	*Poecilia reticulata*	Actinopterygii	non-IVF	growth	performance	17	0.650
36	Reynolds and Gross 1992	*Poecilia reticulata*	Actinopterygii	non-IVF	reproductive	performance	14	0.650
37	Woodward 1986	*Scaphiopus multiplicatus*	Amphibia	non-IVF	development	performance	12	0.152
38	Woodward 1986	*Scaphiopus multiplicatus*	Amphibia	non-IVF	growth	performance	12	0.262
39	Woodward 1987	*Scaphiopus couchi*	Amphibia	non-IVF	adult size	performance	16	-0.200
40	Woodward 1987	*Scaphiopus couchi*	Amphibia	non-IVF	development	performance	16	-0.119

No	Reference	Species	Class	Breeding scheme	Offspring trait	Offspring trait type	Number of sires	r
41	Woodward 1987	*Scaphiopus couchi*	Amphibia	non-IVF	growth	performance	16	0.094
42	Woodward 1987	*Scaphiopus couchi*	Amphibia	non-IVF	developmental	performance	16	-0.320
43	Hoefler et al. 2009	*Pardosa milvina*	Arachnida	non-IVF	development	performance	28	0.539
44	Watson 1998	*Neriene litigiosa*	Arachnida	non-IVF	growth	performance	12	0.657
45	Hadfield et al. 2006	*Parus caeruleus*	Aves	non-IVF	growth	performance	213	-0.150
46	Hadfield et al. 2006	*Parus caeruleus*	Aves	non-IVF	growth	performance	213	0.050
47	Petrie 1994	*Pavo cristatus*	Aves	non-IVF	growth	performance	8	0.660
48	Petrie 1994	*Pavo cristatus*	Aves	non-IVF	growth	performance	8	0.740
49	Boake 1985	*Tribolium castaneum*	Insecta	non-IVF	development	performance	32	-0.340
50	Boake 1985	*Tribolium castaneum*	Insecta	non-IVF	reproductive	performance	32	-0.100
51	Boake 1985	*Tribolium castaneum*	Insecta	non-IVF	reproductive	performance	32	-0.320
52	Kotiaho et al. 2001	*Onthophagus taurus*	Insecta	non-IVF	condition	performance	12	0.709
53	Leibowitz et al. 1995	*Drosophila buzzatii*	Insecta	non-IVF	developmental	performance	210	0.130
54	Wilcockson et al. 1995	*Coleopa frigida*	Insecta	non-IVF	adult size	performance	136	0.272
55	Byers and Waits 2006	*Antilocapra americana*	Mammalia	non-IVF	growth	performance	12	0.623
56	Kruczek & Zatorska 2008	*Myodes glareolus*	Mammalia	non-IVF	physiological	performance	34	0.499
57	Kruczek & Zatorska 2008	*Myodes glareolus*	Mammalia	non-IVF	physiological	performance	34	0.677
58	Kruczek & Zatorska 2008	*Myodes glareolus*	Mammalia	non-IVF	physiological	performance	33	0.756
59	Arellano-Aguilar and Macias Garcia 2008	*Girardinichthys multiradiatus*	Actinopterygii	non-IVF	display	sexually selected	5	0.502
60	Arellano-Aguilar and Macias Garcia 2008	*Girardinichthys multiradiatus*	Actinopterygii	non-IVF	display	sexually selected	5	0.959

No	Reference	Species	Class	Breeding scheme	Offspring trait	Offspring trait type	Number of sires	r
61	Bakker 1993	*Gasterosteus aculeatus*	Actinopterygii	non-IVF	display	sexually selected	6	0.889
62	Brooks 2000	*Poecilia reticulata*	Actinopterygii	non-IVF	attractiveness	sexually selected	36	0.612
63	Houde 1992	*Poecilia reticulata*	Actinopterygii	non-IVF	display	sexually selected	37	0.626
64	Karino & Hajima 2001	*Poecilia reticulata*	Actinopterygii	non-IVF	display	sexually selected	47	0.309
65	Karino & Hajima 2001	*Poecilia reticulata*	Actinopterygii	non-IVF	display	sexually selected	35	0.294
66	Karino & Hajima 2001	*Poecilia reticulata*	Actinopterygii	non-IVF	display	sexually selected	35	0.628
67	Karino & Hajima 2001	*Poecilia reticulata*	Actinopterygii	non-IVF	adult size	sexually selected	47	0.634
68	Reynolds and Gross 1992	*Poecilia reticulata*	Actinopterygii	non-IVF	adult size	sexually selected	17	0.600
69	Aspi & Hoikkala 1993	*Drosophila littoralis*	Insecta	non-IVF	display	sexually selected	85	0.000
70	Aspi & Hoikkala 1993	*Drosophila littoralis*	Insecta	non-IVF	display	sexually selected	85	-0.016
71	Aspi & Hoikkala 1993	*Drosophila littoralis*	Insecta	non-IVF	display	sexually selected	60	0.079
72	Aspi & Hoikkala 1993	*Drosophila littoralis*	Insecta	non-IVF	display	sexually selected	60	0.193
73	Aspi & Hoikkala 1993	*Drosophila montana*	Insecta	non-IVF	display	sexually selected	81	0.086
74	Aspi & Hoikkala 1993	*Drosophila montana*	Insecta	non-IVF	display	sexually selected	81	0.124
75	Aspi & Hoikkala 1993	*Drosophila montana*	Insecta	non-IVF	display	sexually selected	56	0.224
76	Aspi & Hoikkala 1993	*Drosophila montana*	Insecta	non-IVF	display	sexually selected	56	0.328
77	Boake and Konigsberg 1998	*Drosophila silvestris*	Insecta	non-IVF	display	sexually selected	117	0.096
78	Boake and Konigsberg 1998	*Drosophila silvestris*	Insecta	non-IVF	display	sexually selected	117	0.124
79	Bounduriansky and Rowe 2005	*Prochyliza xanthostoma*	Insecta	non-IVF	adult size	sexually selected	58	0.421
80	Collins et al. 1999	*Achroia grisella*	Insecta	non-IVF	display	sexually selected	47	0.232

No	Reference	Species	Class	Breeding scheme	Offspring trait	Offspring trait type	Number of sires	r
81	Collins et al. 1999	*Achroia grisella*	Insecta	non-IVF	display	sexually selected	47	0.408
82	Collins et al. 1999	*Achroia grisella*	Insecta	non-IVF	display	sexually selected	47	0.416
83	Collins et al. 1999	*Achroia grisella*	Insecta	non-IVF	display	sexually selected	33	0.065
84	Collins et al. 1999	*Achroia grisella*	Insecta	non-IVF	display	sexually selected	33	0.254
85	Collins et al. 1999	*Achroia grisella*	Insecta	non-IVF	display	sexually selected	33	0.508
86	Day et al. 1996	*Coleopa frigida*	Insecta	non-IVF	adult size	sexually selected	136	0.625
87	Day et al. 1996	*Coleopa frigida*	Insecta	non-IVF	adult size	sexually selected	100	0.337
88	Day et al. 1996	*Coleopa frigida*	Insecta	non-IVF	adult size	sexually selected	97	0.275
89	Day et al. 1996	*Coleopa frigida*	Insecta	non-IVF	adult size	sexually selected	86	0.371
90	Day et al. 1996	*Coleopa frigida*	Insecta	non-IVF	adult size	sexually selected	81	0.317
91	Edvardsson and Arnqvist 2006	*Tribolium castaneum*	Insecta	non-IVF	display	sexually selected	24	-0.041
92	Gromko 1987	*Drosophila melanogaster*	Insecta	non-IVF	display	sexually selected	330	0.045
93	Gromko 1987	*Drosophila melanogaster*	Insecta	non-IVF	display	sexually selected	330	0.030
94	Leibowitz et al. 1995	*Drosophila buzzatii*	Insecta	non-IVF	adult size	sexually selected	210	0.061
95	Polak et al. 2004	*Drosophila bipectinata*	Insecta	non-IVF	display	sexually selected	15	0.627
96	Polak et al. 2004	*Drosophila bipectinata*	Insecta	non-IVF	display	sexually selected	8	0.812
97	Ritchie and Kyriacou 1994	*Drosophila melanogaster*	Insecta	non-IVF	display	sexually selected	44	-0.075
98	Ritchie and Kyriacou 1994	*Drosophila melanogaster*	Insecta	non-IVF	display	sexually selected	44	0.043
99	Tomkins & Simmons 1999	*Forficula auricularia*	Insecta	non-IVF	display	sexually selected	20	0.486
100	Wilcockson et al. 1995	*Coleopa frigida*	Insecta	non-IVF	adult size	sexually selected	136	0.625
101	Horne and Ylonen 1998	*Myodes glareolus*	Mammalia	non-IVF	dominance	sexually selected	166	0.257

No	Reference	Species	Class	Breeding scheme	Offspring trait	Offspring trait type	Number of sires	r
102	Mills et al. 2007	*Myodes glareolus*	Mammalia	non-IVF	dominance	sexually selected	35	-0.342
103	Mills et al. 2007	*Myodes glareolus*	Mammalia	non-IVF	dominance	sexually selected	42	0.094
104	Mills et al. 2007	*Myodes glareolus*	Mammalia	non-IVF	dominance	sexually selected	36	0.417
105	Oksanen et al. 1999	*Myodes glareolus*	Mammalia	non-IVF	mating success	sexually selected	52	0.281
106	Brooks 2000	*Poecilia reticulata*	Actinopterygii	non-IVF	survival	viability	39	-1.359
107	Brooks 2000	*Poecilia reticulata*	Actinopterygii	non-IVF	survival	viability	39	-0.306
108	Brooks 2000	*Poecilia reticulata*	Actinopterygii	non-IVF	survival	viability	39	-0.049
109	Woodward 1987	*Scaphiopus couchi*	Amphibia	non-IVF	survival	viability	12	0.417
110	Alatalo et al. 1998	*Hygrolycosa rubrofasciata*	Arachnida	non-IVF	survival	viability	71	0.203
111	Hadfield et al. 2006	*Parus caeruleus*	Aves	non-IVF	recruitment	viability	77	0.115
112	Hadfield et al. 2006	*Parus caeruleus*	Aves	non-IVF	recruitment	viability	77	-0.042
113	Petrie 1994	*Pavo cristatus*	Aves	non-IVF	survival	viability	8	0.596
114	Petrie 1994	*Pavo cristatus*	Aves	non-IVF	survival	viability	8	0.840
115	Van Schantz et al. 1994	*Phasianus colchicus*	Aves	non-IVF	survival	viability	17	0.109
116	Edvardsson and Arnqvist 2006	*Tribolium castaneum*	Insecta	non-IVF	survival	viability	24	-0.288
117	Edvardsson and Arnqvist 2006	*Tribolium castaneum*	Insecta	non-IVF	survival	viability	24	0.199
118	Byers and Waits 2006	*Antilocapra americana*	Mammalia	non-IVF	survival	viability	45	0.172
119	Byers and Waits 2006	*Antilocapra americana*	Mammalia	non-IVF	survival	viability	45	0.423

Appendix – References

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